

Better treaty is worth waiting for

JULES VERNE in *Sens dessus dessous* (1889) reported that the government in Washington was very interested in the possibility of firing a projectile of 180,000 tons which would displace the North Pole by 23°, thus eliminating the obliquity of the ecliptic and thereby seasonal variations in the Sun's elevation. During 1956, Senator Estes Kefauver, the US Vice-Presidential candidate declared that hydrogen bomb tests could tilt the axis by 10°. Neither Verne's nor Kefauver's calculation took into account the Earth's spheroidal shape. When this is accounted for, the displacements in both cases assume more modest dimensions—being reduced by a factor of about 10¹². "After seventy years", wrote Walter Munk and Gordon Macdonald, in telling this story in *The Rotation of the Earth* (Cambridge University Press), "the government in Washington still refuses to recognise the existence of the equatorial bulge".

This week the United Nations Conference of the Committee on Disarmament reconvenes in Geneva, and environmental warfare will be high on the list of topics to be studied. If denying Moscow (and London) a decent summer every year is less high on the list these days, there still remain plenty of possibilities, however unfeasible they may be at present. They include

- stimulation or suppression of rain, hail, fog, snow and lightning
- generation and guidance of tornadoes and hurricanes
- modification of climate
- diversion or pollution of rivers and ocean currents
- changes in physical, chemical or electrical properties of the atmosphere or oceans
- stimulation of earthquakes and/or oceanic tidal waves
- disruption of natural vegetative cover

Excellent surveys are given by Jozef Goldblat and Bhupendra Jasani in a recent issue of *Ambio* (Vol. IV, No. 5-6).

In the past two years the United States and Soviet Union have been holding bilateral talks on environmental modification, and will be proposing a draft convention. Its crucial sentence runs: "Each State Party to this Convention undertakes not to engage in military or any other hostile use of environmental modification techniques having widespread, long-lasting or severe effects as the means of destruction, damage or injury to another State Party". Environmental modification techniques are later defined to comprise techniques which change the environment "through the deliberate modification of natural processes".

A veritable industry has grown up around the analysis of what is omitted from arms control and disarmament measures, and this convention offers particularly rich pickings. The exercise is helped by the existence of two earlier drafts—one proposed by liberals in the United States Senate in 1973 and given overwhelming support there, the other submitted to the United Nations General Assembly in 1974 by the Soviet Union. The table shows the most significant variations.

The omissions show rather clearly US military thinking on the subject. Environmental modification techniques that

	Military R & D forbidden?	All states protected?	All operations forbidden?	Review Conference after 5 years?
US Senate Resolution (1973)	Yes	Yes	Yes	Yes
USSR Proposal (1974)	'Prepara- tions for use' for- bidden	Yes	Yes	Yes
US/USSR Proposal (1975/6)	No	No, only 'States Party'	No, only those lead- ing to 'widespread, long-lasting or severe effects'	No

are limited in extent, short in duration or not too severe are permitted. Bickering about where the line is drawn is, by the convention, transferred to the Security Council, where the veto can be used. Research and development are permitted, so techniques can be brought to the point of readiness; if any country decides to turn its hostility on another and has a new and remarkable weapon it is unlikely to be too squeamish about ignoring a treaty. It is difficult to know whether to attach any significance to the limitation of beneficiaries to 'States Party'. China has not been in the habit of signing treaties; the two superpowers seem to be keeping their options open in her direction. Finally, the lack of a review conference seems to be the easiest way to allow the subject to go underground once the ink is dry on the treaty.

The definition of environmental modification as change "through the deliberate modification of natural processes" was not in the earlier drafts. Was it added simply for neatness or does it exclude anything significant? It does resolve an ambiguity, namely, whether bombing a dam to flood the countryside or illuminating enemy territory at night time is to be regarded as environmental modification. Unfortunately, as with so much else in the convention, the almost feasible is declared beyond its scope.

There are many reasons why the proposed convention is weak, and since widespread, long-lasting and severe modifications are by no means yet upon us (indeed many ideas are by common consent, fairly ridiculous) there is still scope for action. The superpowers should be told to go away and work out something more meaningful in which research and development for peaceful purposes is given a firm international grounding, since the environment does not recognise frontiers. That done, there need be no excuse that military research and development is needed because it might have peaceful applications. And a much more realistic treaty could be written. □



Australian aftermath

Political changes have affected Australian science. In the first of two articles. **Peter Pockley** paints a picture of the period up to Labour's downfall.

WHILE it may be rash to write historical perspectives close to the events under examination, a claim that Australian science politics have never had a more turbulent twelve months would be hard to beat. The dismissal of Mr Gough Whitlam's Labour government by the Governor General, Sir John Kerr, on 11 November and its replacement, first by vice-regal fiat and ultimately by election of 13 December, by the conservative coalition led by Mr Malcolm Fraser has brought about an atmosphere of tight security and public silence on most areas of government policies and practices. Yet, science politics have boiled merrily along in the public gaze.

All government departments with a substantial science component have suffered change, not because of their scientific responsibilities but largely as a by-product of Mr Fraser's determination to dismantle many of Labour's bureaucratic monuments. However, while the new Prime Minister is working hard to do this, or simply to neglect Labour's social initiatives, like medical and legal aid, he will be hard put to affect one over-riding legacy of the three Whitlam years. This is the evident politicisation of many sectors of Australian society which had previously never noticed, let alone used, their political feet. Under the previous 23 years of Liberal-Country Party rule, the most successful influences on government were the established forces of commerce who operated largely in a private, sometimes covert, fashion. Under Labour, the opportunities for overt political action were expanded, partly by design, partly by default.

The environmentalists and scientists, for example, were encouraged into political action by the deliberate design of direct channels to Cabinet through the establishment of ministers and departments devoted to their interests. It should be noted, though, that these two particular groups and ministries were vastly different. The Environment Department, begun from scratch, had an activist and intellectual in Dr Moss Cass as its first Minister. The staffing of the department had a strong scientific basis from the Secretary, Dr Don McMichael, down. The environmental movement was among the strongest supporters of Labour in their 1972 and 1974 election victories.

In contrast, the Science Department

was formed by splitting the former Department of Education and Science, whose head, Sir Hugh Ennor, went to the Science part. Despite the new organisational design, Australian scientists were slow to forge a stronger platform for their own interests. Some blame was directed at the department which, apart from Sir Hugh Ennor, a former biochemistry professor at the Australian National University, was not strong in scientific experience. The new Minister for Science, Mr Bill Morrison, was less encouraging to the scientists and bureaucrats under his control than he might have been, both through his critical approach to their work and a certain lack of energy in publicly promoting their interests. In an interview for *Nature* in 1973, Mr Morrison remarked that "there are absolutely no votes in science": if true then his time as Science Minister was politically useless to him, but he only needed 30 of the votes which went to his Liberal opponent to have saved his seat at the recent election, and there just might have been 30 disinherited scientists in his electorate.

Two confrontations

When the government defaulted seriously on science matters, though, the general political climate had prepared scientists for exerting effective public pressure. After a constructive start to 1975 with the announcement in January of the formation of the Australian Science and Technology Council (ASTEC), the government had to ride out two major confrontations with the scientific community in which the scientists displayed considerable nous and had significant effect. The first bolover was the raid on CSIRO's statutory responsibilities by the then Minerals and Energy Minister, Mr Rex Connor (who was returned at the recent poll), following the extraordinary scene of Mr Clyde Cameron's fit of pique in refusing for a time to serve in the Science portfolio. In the face of heavy odds, CSIRO held its ground in the fight.

The second occasion concerned a first-class foul-up of the financing of research grants. There are two principal government schemes for supporting individual and small group research; these are run by the Australian Research Grants Committee (ARGC) and the National Health and Medical

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The old, the new and the ever-present: Gough Whitlam (top), Malcolm Fraser (centre) and Sir John Kerr (bottom). (Photos: AIS)

Research Council (NH&MRC). The funds available to these schemes were ruthlessly slashed in the August budget; ARGC's funds for 1976 were cut by 66% to about \$3 million, and NH&MRC's by 43% to about \$4 million. The exact amounts were hard to ascertain from the budget papers which referred to the financial year 1975/76 (July to June) while the research grants schemes, like universities, conformed to the calendar year. The reality of the potential disaster to research in universities and non-government institutions only sank slowly into the collective consciousness of Australian scientists.

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'The disparate and dispersed nature of Australia's scientific community normally militates against unified and forceful action on a national basis. But, although slow in ignition, the scientists were long in burning'

were long in burning. In the face of an inflation-conscious government, resolute at last to hold the line on overall expenditure, the scientists mobilised themselves with nationwide protest meetings and lobbying through the press and private channels; one remarkable occasion in Melbourne drew 800 odd researchers—the atmosphere was described as “euphorically united” against the philistine forces of government. By late September, they had forced both Ministers responsible (Science and Consumer Affairs—Mr Cameron — and Health — Dr Doug Everingham, who was later defeated at the poll) to admit to bungling through a failure to appreciate the difference between financial and calendar years during the final budget negotiations. Face-saving formulae were found to restore the 1976 grants to roughly the 1975 levels in paper money terms; for 1976, ARGC finally allocated \$7.2 million and NH&MRC \$7.15 million. Even these levels, though, constituted about 12–15% less in real terms than the previous year due to the effects of inflation.

Prelude to the fall

By the time the research grants controversy had died down, the Labour Government was beginning to look decidedly shaky. Following the mid-year Cabinet reshuffle, there was firmer and more competent leadership among the senior ministers, but their economic decisions, although basically sound, were biting hard on many important groups. The budget was severe on the

19 universities and 85 advanced colleges by deferring triennial financing for one year. A “pause year” was introduced for 1976, before resumption of the progression of triennia in 1977. The recommendations of the Universities Commission and the Commission on Advanced Education, for expenditure of \$1,780 million and \$1,681 million for the 1976–78 triennium, were set aside, and higher education was thrown into great uncertainty. The effects, though, were mainly felt by the senior administrators of universities and colleges and even their public protests did not cut much ice among their staffs.

The consequences of the economic situation and of Labour's administration of it did not strike home to the great bulk of academics until their own personal interests in research were threatened. In comparison with the cutbacks in growth of expenditure on salaries and capital, the initial research cuts were very small beer (for universities the cutback is of the order of \$200 million for 1976, an exact figure being hard to calculate on a comparative, annual basis because of triennial financing). Yet those initial research cuts, totalling about \$7 million, did more to lose the confidence of the academic community in the government than any other factor, and the government could ill afford to lose the support of any articulate group. Ironically, when the constitutional issue reached crisis point, many of the previously disenchanted academics flocked back to active support of the Labour cause.

It is doubtful if the scientists of Australia ever saw themselves as the precipitants of crisis in government, but their associations with key political events of 1975 were close and, at times, uncomfortably so. By being forced into political defence of their own territory, many scientists appeared to become more informed and articulate on basic political matters than previously.

Mr Fraser chose to use his majority in the Senate to defer Labour's Budget after Mr Whitlam had sacked Mr Connor from the Ministry for allegedly misleading Parliament on an aspect of the long-running “overseas loans affair”. Mr Fraser also took one or two other useful precautions before embarking on this unprecedented course—like squaring off the proprietors of the three national chains of newspapers, whose unquestioning support he enjoyed throughout the campaign.

With the fall of Mr Connor, his adviser Professor Harry Messel disappeared from immediate view in Canberra. The other member of Mr Connor's tightly knit team in the raid on CSIRO, Sir Lennox Hewitt, then Secretary of Mr Connor's Department,

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Top: Sir Hugh Ennor, Secretary, Department of Science. Below: Rex Connor, former Minerals and Energy minister. (Photos: AIS)

had already departed the Canberra scene, having been strongly encouraged to move sideways into the security of the chairmanship of Qantas, Australia's overseas airline. Nonetheless, the awkward division of responsibility for CSIRO between the Minister for Science and Consumer Affairs and the Minister for Minerals and Energy was translated into a division of CSIRO's funds (\$143 million from all sources for 1975/76) along similar lines in the August budget. Since, however, the budget did not get passed by the Senate until the election had been forced on Labour by the Governor General, the personality of CSIRO was never effectively split by these arrangements.

(to be concluded)

USSR

Plyushch says protest

The Soviet Union recently released Leonid Plyushch, the Ukrainian mathematician held for over three years in Dnepropetrovsk penal mental hospital. Vera Rich reports

"I consider that the scientists of the west should protest in an organised manner and publicly against the campaign in the Soviet Union against dissidents, and against prisons, camps and penal psychiatric institutions". This statement has come from Leonid Plyushch, who warmly acknowledges the help of the western academic community in effecting his release from the hospital following the granting of an exit visa in January. He told *Nature* that he thought pressure from Western scientists was "one of the active factors which assisted my release".

In Plyushch's case, a two-pronged attack on the Soviet authorities was mounted on his behalf—on the one hand by mathematicians and cyberneticists (his professional colleagues), and on the other by psychiatrists, who saw his continued incarceration and the massive doses of drugs to which he was subjected as a blot upon their profession. The mathematicians' campaign followed what has by now become a standard pattern—appeals to the Soviet authorities, proposals to elect Plyushch as a member of eminent mathematical bodies and a concerted effort to keep his case before world opinion. The psychiatrists' campaign was a more complex matter. They were not only concerned with effecting Plyushch's release; they also felt that his committal impugned the honour of their profession. While working to secure his release, they therefore sought a reversal of the diagnosis. This diagnosis stated, apparently on the basis of his criticism of Soviet "state capitalism" (Plyushch's term), his defence of his native Ukrainian culture, and his signature on a petition to the United Nations, that he was suffering from "sluggish schizophrenia" and "reformism"—diseases which, as yet, the psychiatric profession elsewhere does not acknowledge.

Plyushch himself has offered two possible reasons for his committal to the *psikhushka* rather than to an ordinary prison or camp: firstly, he refused to answer the interrogators' questions, "because I considered, and I told them so, that the KGB is an anti-Soviet, anti-Communist, anti-constitutional organisation"; secondly, "because they knew that in court I

would speak from a Communist position and that nothing would happen in court to show that I was an anti-Soviet person". Since it appears to be current Soviet policy to present all separatism in the Union Republics as inspired by fascism and international capitalism, this would have been extremely embarrassing.

Plyushch's ideal for his native Ukraine is an independent socialist state on the Dubcek model—or, at the very least, a reversal of the policy of Russification of higher education and learning, which since the decree on education of November 1958 has gradually introduced Russian as the universal language of the learned professions throughout the Soviet Union. This has in some cases reduced formerly important learned journals into little more than overflow outlets for Moscow publications, and has undermined the very promising lead which, fifteen years ago, the Ukrainian SSR was making in Plyushch's own field of cybernetics.

Soviet response

In belated response to the campaign by bodies like the Royal College of Psychiatrists, the Soviet authorities, in the person of Dr Snezhnevskii of the Serbskii Institute of Psychiatric Medicine in Moscow, countered finally with accusations of non-professional conduct against the Royal College, which had mentioned "patients" by name. This particular correspondence between the Royal College and Snezhnevskii has not yet been concluded, but it is worth noting that the College only voiced its protests after appeals to world opinion from Plyushch's family and the families of other "psychiatric" prisoners; moreover, an official at the Soviet embassy in Paris had himself divulged names of internees (including that of Plyushch) when he claimed (in a communication to the French mathematician Henri Cartan) that Western psychiatrists who were shown the relevant documents at the Serbskii Institute had all agreed that the persons in question were ill at the time of detention (each of the psychiatrists concerned, who included Dr Denis Leigh, the Secretary General of the World Psychiatric Association, and an ex-President of the American Psychiatric Association, publicly denied this later).

With Plyushch's release, the Soviet Embassy in London has made a determined stand to prove that, even if Plyushch were now sane (as three

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Photo: AP

Leonid Plyushch: released

Western psychiatrists—Drs Low-Beer, Sluga and Ferdiere—have declared), he was at the time of his committal mentally ill. *Soviet News*, the official embassy "hand-out", presents his case as a cure; his mental health is said to have "improved lately", "as a result of treatment". His release is thus presented as a triumph for Soviet psychiatry. To reinforce this impression *Soviet News* also carries an interview with Denis Leigh. General in its content, in its present context it could be misconstrued as supporting the Soviet viewpoint on Plyushch.

Although the Plyushch case could now be considered closed, the evidence he has brought with him does reopen certain issues for Western scientists. They have long known of the Soviet use of drugs such as haloperidol, sulfazin and triflazine (stelazin) as a means of repression, and what now emerges from Plyushch's evidence is the number of "patients" involved. Plyushch says that he has "almost no information" about other psychiatric hospitals, and he understands that "things are a bit better" in them. But Dnepropetrovsk, he says, is the worst, and he reports that some 60 dissidents, and not the few originally thought, are at present confined there. He stresses the problem that "in a psychiatric prison, a person degenerates intellectually".

Since it is among the intellectuals that the dissident movement in all its varieties has taken the strongest hold, Plyushch's evidence seems certain to provoke considerable concern and disquiet among the world scientific and academic community as a whole. □

USA

The public's case is put

The tortuous process of setting controls on the use of a revolutionary, but potentially hazardous, technique for manipulating genes from living organisms has entered a new and politically important stage in Washington. Colin Norman reports

For the first time, the question of controls over genetic manipulation experiments was last week opened up to allow members of various public groups to express their opinions on the risks and benefits of the research, and to influence the development of the proposed controls. The forum was provided by an open meeting of a top-level advisory committee of the National Institutes of Health (NIH), the institution which funds the bulk of biomedical research in the United States. The meeting was called by NIH Director Dr Donald Fredrickson to discuss whether or not the proposed guidelines, drafted by another NIH committee last December after months of argument and confusion, should be adopted. Fredrickson will decide that question before the drafting committee next meets in April. Hanging on his decision are several exciting experiments which are currently under embargo.

The technique involves the use of a newly-discovered class of enzymes to transplant genes from one organism into another. The utility of the technique is that it enables genes from any organism, including man, to be inserted into a virus or bacterium so that they are copied by the reproductive machinery of their new host. That possibility could open up revolutionary advances in understanding how genes work and more distantly, it could lead to such applications as improving yields of crop plants and repairing genetic diseases.

But, in 1973, scientists involved in the research began to express concern about the fact that the technique allows genes to be shuffled between species and joined in combinations which are unlikely to have occurred by evolution. It is conceivable, for example, that a virus or bacterium bearing transplanted genes could be endowed with unpredictable biological properties so that, in the worst imaginable case, a novel epidemic could be let loose if they escape from the laboratory. Such concerns led a committee of the National Academy of Sciences, chaired by Paul Berg of Stanford University, to urge in July 1974 that a

moratorium be placed on some uses of the techniques until the hazards are assessed. That call was followed early last year by an international meeting of geneticists to discuss the hazards; since then, the NIH committee has been attempting to draft regulations to control the technique.

Last week's meeting provided a confused discussion which resulted in conflicting advice being given to Fredrickson about the acceptability of the proposed regulations. The committee, which consisted of scientists, lawyers and lay members, listened to statements from those who drafted the regulations and from a variety of groups and individuals, on the basis of which each member will provide Fredrickson with views on whether or not the proposed controls are acceptable. The fundamental issue is whether, and under what conditions, some of the research which was placed under a voluntary moratorium by the Berg committee's appeal should be allowed to go ahead.

The proposed controls would allow some of the research to be resumed, but under very strict conditions. Essentially, the controls specify that most experiments should be performed in laboratories especially equipped with safety devices—in much the same way that research involving hazardous pathogens is now performed—and, in addition, genes should only be transplanted into organisms which have been crippled in such a way that they would be incapable of surviving outside the laboratory.

Berg himself said last week that he believes that the proposed controls are "stricter than necessary to protect public health", but added that he believes that it is better to err on the side of caution. Similarly, Dr Roy Curtiss, a microbiologist from the University of Alabama, told the committee that he believes the proposed controls are sufficiently strict to guard against the possible hazards. He also announced that he has constructed in his laboratory a strain of the common intestinal bacterium *E. coli*, which appears to have been mutated so as to be incapable of surviving outside an artificial laboratory environment. *E. coli*, the geneticists' workhorse, is the most likely host for transplanted genes.

But a few scientists expressed the opinion that the proposed guidelines are too strict, and will greatly restrict an exciting and potentially beneficial area of research. Dr David Hogness, a member of the committee which drafted the proposed controls, for

example, suggested that there is little evidence that the speculated hazards are real, and argued that "in the present climate of opinion, the benefits of increasing knowledge have been underemphasised". And Dr Donald Brown, a geneticist who has been using the technique to grow and purify frog genes in *E. coli*, suggested that the controls are so strict as to be totally irrational.

On the other hand, several other witnesses, most prominently members of a group of radical scientists called the Boston Area Recombinant DNA group, argued that many planned experiments should be delayed, at least until the hazards have been evaluated. In particular, they urged that *E. coli* should not be used as the host for transplanted genes because that bacterium is a common inhabitant of the human gut.

Dr Allen Silverstone, a spokesman for the Boston group said, for example, that "unless we can be assured that the possibility of danger is reduced to insignificance, we would suggest that the NIH withhold funding such research, until the questions of safety and procedure are settled, especially to the satisfaction of honest critics within the scientific community and the public. We do not propose this lightly. We recognise that many scientists wish to do these experiments, and thus far their restraint has been admirable."

Faced with those conflicting views, Fredrickson must soon decide whether or not to accept the proposed controls or suggest that they should be modified. If he does suggest modifications, it is likely that he will require that the drafting committee should pay more attention to devising ways in which the proposed controls should be implemented.

The proposed guidelines, which would apply only to research funded by NIH, suggest that implementation of the controls should largely be left to institutional committees, which would ensure that the safety precautions at institutions where experiments will be undertaken, meet conditions set out in the regulations.

It should be noted that, by providing public input into his decision, Fredrickson has drawn the sting from a common criticism that the process has lacked participation from people outside the scientific community. Senator Edward Kennedy, for example, has suggested that such matters are too important to be left solely to scientists, and he would presumably be ready to provide such input by legislation if necessary. Last week's public meeting should at least guard against the research being regulated by legislation. □

SWEDEN

Probing the ionosphere

Representatives from six European countries met in Kiruna, North Sweden, last month to inaugurate the EISCAT project for research into the Earth's higher atmosphere. Wendy Barnaby reports

THE aim of the \$21 million EISCAT (European Incoherent Scatter Facility) project, which scientific organizations in Britain, Finland, France, Norway, Sweden and West Germany are sponsoring, is to learn more about the chemical, physical and dynamic processes which the solar wind induces in the Earth's ionosphere. The dual frequency high power radar system which will be used is expected to be built in three years' time, and the project will run for at least ten years after that. Britain, France and West Germany will each contribute about a quarter of the initial financing, and the rest will come from the Scandinavians.

As a method of exploring the ionosphere, incoherent scatter involves beaming radar waves there to cause the free electrons of the ionosphere's plasma to operate as antennae and

'scatter' radio signals, which are then monitored by receivers on earth. These signals are rich in information about the properties of the ionosphere and the processes going on within it. The EISCAT facility will operate from VHF (224 MHz) and UHF (933.5 MHz) transmitters at Tromsø, Norway. The signals will be picked up from electrons at heights between about 80 and 2000 km in the ionosphere by a VHF receiver at Tromsø and UHF receivers at Tromsø, Kiruna (Sweden) and Sodankylä (Finland). They will then be passed to the Geophysical Institute at Kiruna, the project's headquarters, for analysis.

Solar-terrestrial physics will undoubtedly be extended by what the analyses will reveal. One member of the EISCAT Council, Dr F. Horner (UK), has explained that the movement of ionization, the density of electrons, the temperature of ions and electrons, structural changes in the ionosphere and neutral atmosphere, and the ionospheric source of the solar wind will all be further clarified. One of the main topics for investigation will be aurori, and the way in which they

are formed by ionization. The facility is being built in high latitudes specifically to make such studies possible. There will be no immediate practical applications of the research, Dr Horner said, although in the long term it will increase understanding of radio communications. It will also throw light on the ways in which the earth's climate is affected by ionospheric conditions.

Although EISCAT is not the first incoherent scatter project, it is by far the most sophisticated. Teams in Peru and Alaska, for example, have already probed the lower ionosphere with radar waves of 1–10 MHz. The British Science Research Council made the last observations in its project MISCAT (multi-static incoherent scatter) as late as last August; this involved the transmission of waves of 400.5 MHz to obtain electron and ion temperatures in a region 100–400 km within the ionosphere. The data obtained is still being processed at the Appleton Laboratory, Slough, and the University College of Wales, Aberystwyth, and is also expected to yield information about the drift velocities of the ions. For the British scientists, therefore, EISCAT is a logical extension of the SRC's work. □

BRITAIN

Engineering a royal society

THE Council of Engineering Institutions (CEI) wheeled on its biggest gun at its tenth anniversary celebration—Prince Philip the retiring president—in the hope of generating badly-needed momentum towards unity in the engineering community.

The Prince, having eloquently exhorted engineers to stick together, launched a new concept—a Fellowship of Engineering, to recognise eminence in the profession; but the idea of a sort of Royal Society for engineers was not without a sizeable band of sceptics.

CEI's problems have multiplied in recent months as engineers, worried about their professional status, their salary, and their public relations, have begun to doubt that CEI was doing enough for them. Meanwhile CEI is suffering from internal strife: its largest members, the Mechanicals, Electricals and Civils, are trying to reform its voting structure, which at present gives equal weight to every one of the fifteen constituent institutions. For lack of progress, the Electricals last December served notice that they would pull out of CEI in a year's time. Unsurprisingly,

the press has now latched on to the woes of CEI in a big way.

Many engineers have looked to CEI to produce some sort of national academy of engineering whose members would be widely recognised as outstanding and whose opinion might be sought by the government. The notion that the government might seek technical advice from professionals not in the Civil Service is a bit outmoded these days, and the need for yet more marks of distinction in engineering doesn't seem entirely obvious when an engineer of distinction can already trail a string of initials like MSc, CEng, FICE, FIMechE, FICGasE, FInstF, MIEE, MIChemE after his name. But FEng is now to be the prize to cap all prizes. Excepting FRS, of course. The Royal Society has been a bit of a problem, because it already has seventy or so on its books with at least nominal engineering qualifications. Proponents of the Fellowship of Engineering want very much the FEng to have equal "status" with FRS; and some would even like the Royal Society to stop electing engineering fellows so as to

prevent FEng from being a sort of failed FRS.

There are others, notably the Mechanicals and Electricals, who also worry about the "failed FRS" image through the association of the Fellowship with CEI, "or any body which may replace it". They argue that CEI or its successor will necessarily be of lower technical status, being "mainly concerned with the mundane day-to-day activities of the average member". They would have preferred the Royal Society to have been more extensively involved, preferably as a co-founder and thus as a guarantor of high standards and continuity. This has not happened; invitations have gone out to the 70 FRS's and to 70 other distinguished engineers to become Fellows of the CEI, and the Royal Society simply says that it has learnt of the initiative with satisfaction.

This new mark of distinction thus suffers at the very beginning from dissent about its parent body. This will take a much more serious turn if the Electricals really do pull out of CEI. There will be anxious searching through CEI's mail these next few days in the hopes that a large fraction of the first 140 (the ultimate number is to be 1000) accept their invitations. □

IN BRIEF

Reactor action

Research and technology ministers from France and Germany discussed plans for the joint development of fast breeder and high temperature reactors at last week's intergovernmental meeting in Nice. Both countries are concerned about rising costs, but their research programmes also complement one another well; moreover plans broke down recently for German cooperation with Britain, whose apparent isolation is beginning to show in the face of co-operation on fast breeders between Germany, Belgium, Luxembourg and the Netherlands.

BNFL setback?

British Nuclear Fuels Ltd (BNFL), still waiting to close the controversial deal to reprocess spent Japanese nuclear fuel at Windscale, is investigating a potentially embarrassing incident there. Last week, radioactive liquid containing tritium, an element capable of diffusing through apparently impermeable materials, seeped into a stainless steel

pipe carrying cooling water through a storage tank that contains reprocessed material. BNFL claim that no radioactivity escaped from the tank.

Safeguards draft

A draft resolution from Britain to fellow members of the International Atomic Energy Agency (IAEA) in Vienna calls for an extension of nuclear safeguards agreements and will, if accepted, come before the IAEA board of governors when it meets next week. Existing safeguards, administered by the IAEA primarily to prevent the clandestine diversion of nuclear materials to military use, apply only to signatories of the Non-Proliferation Treaty; opposition can be expected from non-signatories India and Brazil.

NASA study

A major study released by the National Aeronautics and Space Administration (NASA) says that during the next 25 years space research and development

should be directed more toward Earth-oriented programmes, such as environmental monitoring and weather prediction. The study, conducted by a committee of top NASA officials and outside consultants, may form the basis for much of NASA's planning in the next few years; it recommends that space research should focus more on fundamental problems, such as the evolution of the universe and the nature of black holes, that there should be a major increase in emphasis on data management, and that the USA should develop a permanent space station.

Privacy

The Data Protection Committee, envisaged in the recent UK White Paper on Computers and Privacy as a first step towards a Data Protection Authority, is to be presided over by Sir Kenneth Younger. It was the Younger Committee's report on privacy in 1972 which first recommended an independent review body to oversee the gathering of personal information.

To obtain support for any piece of research today, it is almost essential to claim that one objective is to produce a model. This use of the word in scientific circles is relatively new. Until recently the Shorter Oxford Dictionary restricted its definition to cover such conceptions as small replicas (like Dinky toys) and to describe those persons who posed for artists and photographers, or who displayed *haute couture* garments to society ladies. The date 1971 is given when it included "a simplified or idealised description of a system, situation or process often in mathematical terms, devised to facilitate calculations and predictions" to the Addenda printed at the end of the second volume.

Provided they are correctly used, some models are clearly useful. One of the earliest was Professor Jay Forrester's world model devised for the long-term study of global problems. Provided it was fed with the correct material, it performed admirably. Unfortunately it could easily be misused, as is often indicated in that egregious publication "Limits to Growth". For instance, it is assumed that there is always an increase in environmental pollution if industrial production increases, and so a growth economy must always lead to intolerable pollution and disaster. There are many arguments against uncontrolled economic growth, but this is probably the least valid. Then it is assumed that DDT levels in fish and fish-eating

birds will rise for many years even after the use of DDT ceases. Fortunately it has been found, in several regions, that almost as soon as the

Mistaken models**KENNETH MELLANBY**

use of the insecticide is curtailed, levels in fish-eating birds start to fall. These, however, are misuses of what is clearly a true model, and one that can frequently be useful.

My real complaint is that the word model is so often abused. Many so-called models are no more than descriptions—some could best be described as "maps". We even find a written or spoken description called a "verbal model", though not by me,

nor in any publication for which I have any editorial responsibility. Then there is the mathematical model. I read that "in order that it (a hypothesis) can be tested numerically it has to be stated in mathematical terms: this is what is called a 'model' ". Again, not by me. This so-called model is surely nothing but a mathematical description, with all its faults and advantages. I resent the assumption that this is the only way in which to test a hypothesis. Some of us find a mathematical description, no matter how crude (and such a description can only be an approximation to the truth) illuminates the subject, and gives intellectual satisfaction. Others, and I believe they may be just as good scientists, find such treatment merely confusing. The danger today is that the mathematicians, and even the pseudo-mathematicians, may be gaining in influence, and trying to make others adopt methods which may not be the only ones needed to solve all our problems. All descriptions, mathematical, verbal or conceptual, are inaccurate simplifications of the truth, and can never be a substitute for accurate observation.

Recently model has taken on another meaning. Doorbells in the sleazier parts of London are often labelled simply "Model". Some are more descriptive, as "Second Floor, Lulu, Parisian Model, Very Athletic". This does seem a fruitful field for scientific research.

correspondence

Egypt's needs

SIR,—Egypt's population, as Salah Galal writes (December 18, page 564), may be expected to reach 220 million by the year 2100, and to satisfy the country's water and power needs in the future would call for two Aswan High Dams a year and the equivalent of two extra Nile Rivers. Sea water desalination is looked to as offering the obvious means of supplying the country's water needs.

This presentation of the situation leads me to ask the following questions:

- Does not the projected vast demand for power and water indicate an unreasonable population growth? Perhaps the problem is not so much how to supply the rapidly increasing population as how to keep the population within supplyable limits.

- The product value of 1 m³ of irrigation water in Egypt is about 3 cents (US). The cost of producing 1 m³ of desalinated water by nuclear energy is at least 25 cents. Thus the Egyptian economy will lose 22 cents on every cubic metre of water desalted for use in irrigation, which means a total annual cost of some \$4,000 million for the projected water consumption in the year 2000. Rather than dismaying Egypt's agricultural economists, does this prospect not indicate that a solution to the country's water problem should be sought through other avenues?

As I see it, the most practical answer lies in the more efficient use of the waters of the Nile. It is a fact that a few hundred kilometres east of the Nile Delta, in Israel's northern Negev, the productivity of irrigation water is six times more than in Egypt; the product value per cubic metre of water in that area is 18 cents. Based on this, it may be concluded that the unused potential of the Nile River is itself worth several Niles; and although in order to fully exploit this potential a considerable capital investment might be required, this investment would still be far less than that required for water desalination.

Should it be decided to approach the problem of future resources first and foremost by stepping up the productivity of present resources, I would like to suggest one practical way of contributing towards a solution which would not involve Egypt in inordinate expense: that some of the water draining

from irrigated lands into the sea in the Delta area be sold to Israel.

Every year approximately 10,000 million cubic metres of Egypt's used irrigation water flows through drainage canals out to sea. This water is by Israeli standards still suitable for irrigation. About a tenth of the estimated discharge—equal to the output of dozens of desalting plants—could be absorbed economically in the Israeli Negev and transported there inexpensively by a canal built along the coastal strip of the Sinai Desert. The conveyance of this water to Israel for sale means Egypt's recovery of water that would otherwise be lost.

Egypt's benefits from the transaction would, among other things, be:

- payment for water which at present brings in no economic returns, and
- a guaranteed amount of food production (in the area in question, with modern irrigation methods, one cubic metre of water produces 10 kilograms of potatoes).

The political climate in the region at present might not appear conducive to the implementation of a project of this nature and, for the time being, the idea put forward here may seem fanciful (although perhaps less fanciful than the projected annual 18,000 million cubic metres of desalinated water by the end of the century). However, even at this stage it might be worth examining the economic advantages of such an undertaking for both participants, since perhaps the very consideration of such a scheme could contribute towards the improvement of the political conditions and help to bring closer a time when such a project might become practicable.

Yours faithfully,

E. KALLY

Photochemical smog

SIR,—I wish to call attention to a potential hazard in controlling photochemical smog by reducing hydrocarbon (HC) emissions without a corresponding reduction in the emissions of the oxides of nitrogen (NO_x). If HC concentrations are reduced to keep hourly oxidant levels below the EPA ambient air quality standard of 80 p.p.b., and if NO_x concentrations are not correspondingly reduced, then on many days all the NO will not be oxidized. In the presence of NO, ozone levels are

always considerably below the normal background level of 25 p.p.b., because of the rapid reaction between NO and O₃. Thus on the days that NO is not completely oxidized, ozone levels will be considerably below background levels. If many such days should occur in succession, the bacteria count might increase, and this might enhance the incidence of disease (for example, streptococcus salivarius shows 90% mortality when exposed to 0.025 p.p.m. O₃ at 60–80% relative humidity for 30 minutes).

In many communities automobiles account for a greater percentage of the photochemically active HC than of the NO_x. (In Los Angeles it is about 90% for photochemically active HC, as against 70% for NO_x.) However the control devices presently being installed on cars are designed to control 80% of the HC and 40% of the NO_x. As a result the percentage reduction in photochemically active HC should be about 2–3 times as great as the percentage reduction in NO_x. This may not be a policy of wisdom.

Yours faithfully,

JULIAN HEICKLEN

Pennsylvania State University,
University Park,
Pennsylvania 16802, USA

Turkish poppy industry

SIR,—A footnote to Peter Collin's article (January 15, page 73): when the Government of Turkey forbade farmers to grow poppies, it became essential to collect substantial seed samples of populations of the many types of poppies grown in the country, both to conserve genetic diversity for future use and to ensure that sufficient seed would be available if it were once again to be required. This was successfully organised by the Crop Research and Introduction Centre at Menemen, Izmir, which was established in 1964 by the Government of Turkey with the cooperation of UNDP and FAO, and whose purposes have always included the conservation and study of the vast resources of genetic diversity in the economic plants of Turkey and their wild relatives (see *Nature*, **258**, 278–279; 1975).

Yours faithfully,

A. H. BUNTING

Plant Science Laboratories,
University of Reading, UK

news and views

Immunity to parasites

from K. N. Brown

In a world where a majority of the human population suffer serious morbidity and mortality from eukaryotic parasites, control of these infections must be one of the most pressing of current biomedical problems. Fortunately, there are few more intriguing biological challenges than the capacity of protozoan and metazoan parasites to maintain an integrated existence with their hosts long after being recognised and responded to by the host's immune system. Since they combine this characteristic with an ability to move, as a normal part of their life cycle, between a number of quite different environments, for example, from human blood to mosquito gut, and from fresh water to vertebrate tissue, eukaryotic parasites display an unusual level of phenotypic adaptability.

In the relationship with their vertebrate hosts, parasitic protozoa have the important advantage that every cell (except individual gametes) has the potential to carry the life cycle through to completion. Thus a population can sustain considerable cell death and yet survive as a 'strain'. Because transmission is often intermittent and seasonal however, it is simply not enough for a few individuals to survive host immune reactions, the survivors must themselves multiply in that same immunocompetent environment. In at least three genera, trypanosomes, plasmodia and babesia, this feat is achieved by repeated changes of exposed antigens.

Cross (Parasitology, **71**, 393; 1975) in a careful study of trypanosome variable glycoprotein antigens, has shown very extensive differences in amino acid composition from one variant to another, indicating that, as has long been suspected, variation is a phenotypic event, probably antibody-induced. That differences in the exposed part of the molecule would need to be sufficiently profound to exclude antibody cross-reactivity, could be surmised from the nature of the infection and earlier serological studies, but that they should involve a major part of the molecule came as a surprise.

Whether or not antigenic variation in *Plasmodia* and *Babesia* occurs by changes in analogous surface glyco-

proteins important for extracellular survival, is conjectural, but seems very likely from recent observations by Miller *et al.* (*J. Immun.*, **114**, 1237; 1975).

In any event it is by antigenic variation that these parasites evade some of the worst consequences of the host immune response, and render artificial immunisation extremely difficult. Immunisation by controlled infections still remains the only realistic method of inducing protection, although frequently unsatisfactory and impractical. Thus the recent report of the protective effect of intravenous BCG against murine *Babesia* and *Plasmodia* by Clark *et al.* (*Nature*, **259**, 309; 1976) is important, although the authors' suggestions concerning the role of non-antibody T cell products in destroying intra-erythrocytic parasites, will require much more critical examination before it is generally accepted. The effects, immunological and otherwise, of BCG on host responses to these infections, need investigation.

Many observations have shown that T cells are crucial for protective immunity to malaria. Brown (*Nature*, **230**, 163; 1971) has suggested one interpretation of their role in relation to clinical immunity and antibody responses to successive antigenic variants, and protection has been transferred to non-immunes by T cells from immune animals (Brown, *Ciba Symp.*, **25**, 36; 1974). Elegant experiments by Jayawardena *et al.* (*Nature*, **258**, 149; 1975) using the T₆T₆ chromosome marker have correlated capacity to produce a protective response to malaria with T cell reactivity.

Parasite survival may be helped by their ability to induce immunodepression, and immune responses to other pathogens may be inhibited (for example see Cox, *Nature* **258**, 149; 1975). This effect seems to be localised to the lymphoid tissue directly exposed to the parasite (Golenser *et al.*, *Protozoology*, **22**, 71A; 1975), and is most readily detected during acute high parasitaemias, although in some particularly virulent trypanosome infections immunodepression may be more generalised. Parasites may produce

nonspecific lymphocyte mitogens (Greenwood and Vick, *Nature*, **257**, 592; 1975). Although responses to unrelated antigens may be depressed, however, specific responses to the primary parasitic infection are extremely active, and the antigenic lability of parasites, rather than shortcomings in host immune responses, seem to account for their survival.

Unlike protozoan populations, individual metazoan parasites cannot sustain any great degree of cell death, but they can maintain intimate contact with host tissues without producing any of the localised cellular reactions which normally characterise the presence of foreign tissues. In schistosomes, profound changes occur in the structure of the parasite surface in the first few hours after skin penetration by the aquatic infective larva. Electron microscopy (Hockley and McLaren, *Int. J. Parasit.*, **3**, 13; 1972) suggests that the absence of host cell reaction depends on continuous membrane activity at the schistosome tegument, especially evident in the first few hours after penetration. Other experiments have shown that antibodies which will kill schistosomes immediately on penetration will not destroy older worms (Clegg and Smithers, *Int. J. Parasit.*, **2**, 79; 1972). The parasite surface is immunogenic, since by injecting TNP-treated schistosomes into infected mice, Ramalho-Pinto *et al.* (*Nature*, **259**, 603; 1976) have shown T cell recognition of surface antigens. Since this response falls later in the infection and cannot be recalled readily by cercarial challenge, it may be significant for survival of adult worms. Immunity to reinfection develops normally in these animals, which is rather puzzling.

Curiously, although adult worms usually avoid immune destruction, many are killed in heavy infections. Schistosomes have host-like antigens, probably glycolipids, associated with their surface, which may mask antigenicity (Clegg, *Ciba Symp.*, **25**, 162; 1974; Smithers and Terry, *Adv. Parasit.*, **14**, 399; 1976). Can the specific T cells demonstrated by Ramalho-Pinto *et al.*, under strong antigenic

stimulus, help B cell responses to these host-antigen-worm membrane associations, to produce antibody-mediated death of adult worms? The semi-auto-immune nature of such a response could require for its initiation a heavy infection with its massive antigenic stimulus.

Clearly in infections with protozoan

and metazoan parasites, the problems of parasite-host integration and host immune responses are complex. A full understanding of interactions and reactions at the interface between parasite and host is essential if we are to be able to interpret and modify host-parasite relationships to man's advantage. □

Colliding beam fusion

from John G. Cordey

THE controlled fusion of the light elements deuterium and tritium has been the main goal of plasma physicists for the past 20 years. The basic problem is one of containing a mixture of deuterium and tritium at a temperature of 10^8 °C for a sufficient length of time so that a significant number of fusion reactions take place. At these temperatures the gaseous mixture is a fully ionised plasma which has to be isolated from metallic walls. The main approach to the containment problem has been to use a strong magnetic field to separate the plasma from the walls. Considerable progress has been made using this technique, and toroidal containment devices of the tokamak type are now being designed, which will have parameters similar to those of future fusion reactors (Rebut, *Phys. Bull.*, **26**, 264; 1975).

The precise conditions for a reactor to break-even on energetic grounds were first given by Lawson (*Proc. Phys. Soc.* **B70**, 6; 1967). For a plasma in thermal equilibrium (that is with Maxwellian distributions of deuterons and tritons) the Lawson criterion requires that the temperature be greater than 5 keV, and the product of density and the containment time (the ' $n\tau$ ' product) be greater than 10^{14} s cm⁻³ for break-even.

The colliding beam torus, discussed by Kulsrud and Jassby on page 541 of this issue of *Nature*, and also by Cordey *et al.* (*Nucl. Fusion*, **15**, 710; 1975) is a toroidal containment device in which the deuterons and tritons, instead of being Maxwellian, are oppositely directed beam-like distributions. These counterstreaming distributions are built up by injecting 60 keV beams of deuterons and tritons into, say, a tokamak device. (This technique of beam stacking has been used for many years in accelerators, at a much lower density of course.)

The mean relative velocity of the two counterstreaming distributions is chosen to be close to the peak of the D.T. fusion cross section at around 120 keV. The resulting fusion power output per

unit volume of the CBT (colliding beam torus), is thereby much larger than that of a conventional fusion reactor with Maxwellian distributions at say a temperature of 10 keV. The conditions for a break-even reactor using the CBT approach are as a consequence far less restrictive. In particular, the $n\tau$ product has only to be greater than 3×10^{12} s cm⁻³ for break-even, rather than the 10^{14} s cm⁻³ demanded by the Lawson criteria for the conventional reactor. Since the energy containment time is thought to scale with the radial dimensions of the device, a small scale experiment demonstrating the efficacy of the CBT approach could be built, whereas a demonstration experiment of the conventional reactor will have to be very large indeed. In fact, the next generation of conventional fusion experiments (such as JET, TFTR) are becoming so large and expensive that in Europe they are having to be built collaboratively.

There are however, it seems to me, several possible difficulties associated with this alternative CBT approach. First, the setting up of the colliding beam configuration requires that the containment time of the thermal plasma is less than the fast ion slowing down time, and that there is no recirculation of the thermal plasma. Although as the authors point out, this first condition is satisfied in present-day tokamaks, the parameters in these machines are very different from those of the CBT. In particular, the mean ion energy is much greater than the electron energy whereas the reverse is the case in tokamaks. In view of the fact that the ion distribution in the CBT is anisotropic, there is a strong possibility that the plasma may suffer from microinstabilities which would reduce the slowing down time of the fast ions. The prevention of recirculation of thermal particles relies on the design of an effective diverter; so far there has been little experimental work on diverters and so this part of the proposal remains somewhat speculative.

Also I have some doubts as to

whether the CBT can be developed into an economic reactor in its present form. The quoted values of Q , the energy amplification factor, of 4-8 are somewhat smaller than the value of 15 which is said to be necessary for an economic fusion reactor system (Conn *et al.*, *Nucl. Fusion*, **15**, 775; 1975). Even if a pure fusion reactor is not possible however, the high power output per unit volume of the CBT means that it would be eminently suitable as a neutron test facility or with a fissionable blanket as a fission-fusion hybrid reactor.

One further problem that is not mentioned in this particular article, but discussed elsewhere by Jassby (*MATT*-1145; 1975), is concerned with the penetration of the injected fast neutrals into the plasma. So that the ions leaving the ion sources can move freely across the containing magnetic field, they are neutralised by a gas cell; on entering the plasma they are then re-ionised by the trapped ions. The mean free path of 60 keV deuterium atoms in a plasma of density n is $(2 \times 10^{15})/n$ cm, this places a restriction upon the density or the radius of the device, either of which may affect its reactor potential.

I think to assess whether this novel idea can be developed into a reactor first requires an experimental demonstration of the setting up of the colliding beam configuration. This could probably be done on an existing tokamak with the injection of a few megawatts of, say, 40 keV ions, which is well within the reach of existing neutral beam technology. The scaling of these experiments would then give a clearer indication of the future potential of the CBT as a reactor. □

Biological safeguards in genetic engineering

from David Sherratt

As well as discussing the types of safeguard needed to eliminate any hazards resulting from genetic manipulation experiments in which genes from potentially any organism can be introduced into and replicated in bacteria, a number of biologists are now attempting to assess experimentally the possible hazards and to develop ways in which they can be minimised (see *Nature*, **258**, 861; 1975). Safeguards fall into two categories—physical containment, which prevents the escape of any possibly hazardous material, and biological containment, which ensures

that any possibly hazardous material can only function in precisely defined conditions unlikely to be found outside a designated laboratory. Suitable physical containment facilities are already available in a few laboratories that handle dangerous pathogens.

Since genetic engineering experiments involve the insertion of the DNA to be cloned into a 'vector' which can then be introduced into a bacterium where it is capable of replication and expression, biological containment can be aimed at both the vector and bacterium. At present the favoured vectors are either derivatives of bacteriophage lambda or one of a number of small plasmids, which consist of duplex circles of DNA able to replicate autonomously within bacteria. The favoured host bacterium is *Escherichia coli*, largely because it has been extensively studied by molecular biologists over the past 20 years.

Unfortunately *E. coli* is a normal inhabitant of the gut, and even though most laboratory strains seem to fare badly when competing with the normal intestinal flora, it has been felt advisable to construct even more 'crippled' strains which not only are unable to grow, or transfer any of their genes in the gut, but are killed by the gut environment. To this end, strains are being constructed that have some or all of the following features:

(1) Nutritional requirements which cannot be met in the intestine, such as requirements for the cell wall constituent diaminopimelic acid and nucleic acid precursors; (2) inability to survive at gut temperature and possession of DNA which is degraded at this temperature; (3) sensitivity to certain gut contents such as bile salts.

The introduction of other 'weakening' genetic mutations to the host bacteria (such as nonsense suppressor mutations which upset normal translation and can markedly inhibit cell growth, and temperature-sensitive mutations) may depend on the particular vector used. Since plasmid vectors and their inserted DNA need to be maintained within the host bacterium (naked DNA soon loses its biological activity outside the cell), it is particularly important that the host/plasmid combination is effectively 'crippled' and the presence on the bacterial chromosome or on the plasmid of additional mutations that render the vector unable to replicate or transfer at 37 °C is desirable.

On the other hand, with lambda as a vector, the DNA can be maintained either within the free virus or within a lysogenic cell, integrated into the bacterial chromosome or in some cases in the plasmid state. If a lambda vector contains potentially hazardous genes it is clearly advantageous for it to be un-

able to lysogenise bacteria; then only the virus (and the free DNA) needs to be 'contained'. A number of lambda mutations are known which reduce the frequency of lysogeny greater than 10⁸-fold. Luckily lambda has quite a narrow host range, probably being unable to infect most intestinal strains of *E. coli*. In addition, a number of mutations can be introduced to minimise its spread through sensitive bacterial populations. For example, Enquist *et al.* (page 596 of this issue of *Nature*) have introduced three nonsense mutations into a lambda vector to ensure that viable phage can only be produced in certain suppressor⁺ bacteria, which it is hoped are not likely to be encountered by chance. Another approach is to propagate the vector on a non-modifying strain of bacteria so that the vector DNA will be restricted and degraded in any bacteria having a restriction system. Lambda derivatives containing these types of safety features are also being developed in Edinburgh, Paris, Stanford and Wisconsin.

Another possible hazard results from the fact that free vector DNA can be taken up and replicated by bacteria. Although this process is rather inefficient, the large-scale experiments necessary to obtain sufficient amounts of cloned DNA for some purposes pose a potential hazard. Consequently it is desirable to maximise the yield of vector per bacterial cell. Several thousand copies per cell of some plasmid vectors can be obtained under particular conditions, whilst lambda mutations that make the host lysis-defective are very useful since they increase the phage yield per cell up to 10 times and moreover the phage remain in the bacterial cell until artificially lysed making collection easier and safer. It is also an advantage for the vector to carry conditional-lethal mutations that prevent it replicating in an accidentally infected host under physiological conditions.

An alternative approach to safe genetic engineering is to develop vectors and host organisms that are neither pathogenic to animals and plants nor comprise part of their normal bacterial flora. The favoured organisms emerging from these studies are *Bacillus subtilis* and certain strains of *Pseudomonas*. These are normal soil inhabitants and grow well in defined media, but although a fair amount is known about the genetics and physiology of *B. subtilis*, little is known about the genetics of its potential vectors. By contrast, some *Pseudomonas* plasmids are quite well characterised, although they suffer from the disadvantage (for safety) of being transferable to and expressed in other strains and species. Also, some species of *Bacillus* and *Pseudomonas* are extremely patho-

genic and so again there is the necessity to construct 'safe' vectors whose accidental transfer throughout populations is eliminated. □

Thyroglobulin mystery solved?

from J. R. Tata

For many, thyroglobulin is simply a convenient "marker" for analytical electrophoresis and gel filtration for calibration of molecular weights of proteins. For a few, thyroglobulin is a unique giant ioglycoprotein (molecular weight of 660,000 for the native protein) made exclusively in the thyroid gland of all vertebrates. Its hydrolysis in the gland causes the release of the two thyroid hormones, thyroxine and tri-iodothyronine, which are themselves quite unusual iodoamino acids, known as iodothyronines. Although numerous proteins can be enzymically or chemically iodinated, it is only in the confines of the thyroglobulin molecule that a few privileged mono- and di-iodotyrosyl residues can couple to form the hormonally active iodothyronines covalently retained within the large polypeptide (Edelhoch, *Rec. Progr. Horm. Res.*, **21**, 1; 1965). Because of the uniqueness of its composition and structure, and the clinical and physiological importance of thyroid hormones, the thyroglobulin molecule has been intensively investigated in the past 40 years. A consequence of this activity has been the controversy that has been generated in the past decade over its subunit structure and heterogeneity. The number of subunits proposed has varied from 2 to 10 with molecular weights ranging from 25,000 to 200,000. Two recent reports now seem to put an end to this controversy.

The first report is based on some elegant work by Gilbert Vassart and his colleagues in Brussels (Vassart *et al. Proc. natn. Acad. Sci. U.S.A.*, **72**, 3839; 1975). It exploits the technique of translating heterologous messenger RNAs in *Xenopus* oocytes, first developed in Gurdon's laboratory (Lane *et al. J. molec. Biol.*, **61**, 73; 1971). The Belgian group had earlier immunologically characterised a 33S RNA extracted from membrane-bound polysomes from ox thyroids as mRNA coding for thyroglobulin (Vassart *et al. Eur. J. Biochem.*, **55**, 5; 1975). What they have now done is to demonstrate by sucrose density gradient centrifugation and gel filtration under denaturing conditions that a "19S" non-iodinated thyroglobulin (corresponding to a protein of 600,000 molecular weight) was

the major immunoreactive product of translation of 33S mRNA in *Xenopus* oocytes. The identity of this peptide with thyroglobulin was confirmed by tryptic peptide analysis which led Vassart's group to propose that thyroglobulin is a dimer of two equal subunits of 300,000 daltons. Evidence obtained from more conventional techniques for a similar large subunit of thyroglobulin also comes from a recent report from Edelhoch's laboratory at the National Institutes of Health (Haeberli *et al.*, *J. biol. Chem.*, **250**, 7294; 1975). Ten years ago Harold Edelhoch proposed that thyroglobulin was composed of four smaller subunits. But the new work from the NIH laboratory, on the basis of equilibrium density centrifugation and gel filtration in 6M guanidinium HCl of guinea pig thyroglobulin, definitely establishes the presence of a subunit of about 300,000 daltons. Two smaller subunits of molecular weights of about 210,000 and 110,000 were also detected, but all three had the same amino acid composition as whole thyroglobulin, the only major difference between them being their different iodine contents. A major reason for the earlier observations of a variety of small subunits, and the failure to detect the large one of 300,000 daltons, may lie in the extreme susceptibility of thyroglobulin to both substrate-specific and nonspecific proteases, with which the thyroid gland is particularly well endowed.

There are some interesting points emerging from the Belgian work on microinjection of *Xenopus* oocytes. Earlier experience with the translation of several foreign RNAs in frog oocytes had shown that oocytes are capable of carrying a variety of post-translational modifications of the newly synthesised polypeptide (Lane and Knowland, *The Biochemistry of Animal Development*, **3**, 145; 1975). The recovery of non-iodinated thyroglobulin is the first evidence of a failure of the oocyte to carry out a post-translational modification of a polypeptide synthesised on a vertebrate mRNA. The recovery from the oocyte of such a large sized subunit of thyroglobulin reveals a striking protection against proteolysis of the polypeptide synthesised on the injected mRNA template, especially as the *Xenopus* oocyte is known to degrade certain proteins injected into it (Dehn and Wallace, *J. Cell Biol.*, **58**, 721; 1973). What is not yet known is whether or not the primary translational product in the oocyte is a "pro-thyroglobulin", or even a "pre-pro-thyroglobulin", in line with the increasing evidence that most, if not all, secretory proteins may be synthesised as larger precursors and then specifically cleaved in the membrane of endoplasmic reticulum. In this context,

it would be interesting to find out if thyroglobulin mRNA is translated on membrane-bound ribosomes of the oocytes as it is in the thyroid cell. It would also be interesting to determine whether the oocyte carries out the post-translational glycosylation of thyroglobulin, especially as this protein is unusual in its carbohydrate composition. Whatever mysteries post-transla-

tional modifications may hold, the work from the Belgian and American laboratories seems to have solved that of the subunit structure of thyroglobulin. The work by the Belgian group is also the first in which the technique of microinjection of RNA into frog oocytes or eggs has been successfully applied to answering questions concerning protein structure. □

The Great Barrier Reef

Geomorphology . . .

from George E. Farrow

At a meeting in London on January 28–29, David Stoddart (University of Cambridge) and Sir Maurice Yonge added the results of the 1973 Great Barrier Reef Expedition to the illustrious series of southern zone expeditions with which the Royal Society has long been associated.

COMPARED with other recent Royal Society expeditions such as the much longer Aldabra Expedition of 1967/8 with its vital 'natural history' emphasis, or with the Solomon Islands Expedition of 1965, there was a singular dearth of faunistic papers—in fact only one out of the 19 presented. The meeting was very much a discussion on the recent geological history of the reefs, particularly in relation to possible Holocene sea-level changes.

The 1973 Expedition was financed jointly by the Royal Society and the Universities of Queensland. It worked deliberately north of Cairns, in view of previous concentration on central and southern sectors of the Great Barrier, notably by the famous 1928/29 Expedition. Seventy islands were mapped, and a flora of 200 species collected from forty—a demonstrably Australian flora with many common pan-tropical plants missing.

The most striking achievement of the expedition must surely have been G. R. Orme's (University of Queensland) use of high resolution seismic profiles to demonstrate clearly the remnant nature of the present-day reefs. This lends strong support to Purdy's recent study of central American reefs which similarly showed that modern reefs inherit their structure from earlier, eroded reef limestones. Many submerged terraces were identified on the Barrier, those at –45 m and –109 m being strongly developed. The latter was associated with a buried valley system, overlying which a thick fluvialite regressive sequence seems

likely.

More than 80 radiocarbon dates have so far been obtained from material collected during the expedition, dating being carried out at the Australian National University, Canberra. Rationalisation of the dates in terms of a 'Fairbridge-type' sea-level curve proved impossible, however, because of difficulties in establishing datum levels for both the present and the past. Uncertainty about the eustatic *versus* cyclonic significance of reef island terraces and ramparts made it clear that a critical reassessment is needed of many existing dates used for sea-level reconstructions in the literature.

Though it is premature to attach significance to the dates, several interesting clusters appeared. Microatolls, some with elevations greater than the highest modern 'ponded' examples, commonly date at around 5,500 BP, the bigger cays around 3,500 BP, with two groups of beach rock at 3,000 BP and 2,000 to 1,500 BP. Even the most mobile sand cays give dates which indicate that the sediment is at least 2,000 yr old. Indeed, one came away from the discussion feeling decidedly palimpsest.

A disappointing aspect of the age dating was that suspected pre-Holocene material recovered from a 30-m hole drilled on Bewick Island proved too recrystallised for Uranium Series age determination.

D. R. Stoddart presented clear evidence, by the remapping of Low Isles (so elegantly mapped by Spender in 1928/9), of the major changes that can overtake islands in a very few years. Consternation overtook several members of the 1928/29 Expedition on hearing that the eroded foundations of their headquarters were mapped in 1973 as part of the intertidal zone! An evolution is to be recognised in the sequence of environments (and therefore in the sediments) that results from the diachronous leeward progres-

sion of belts of mangrove, themselves initially dependent on a degree of stability imparted by storm ramparts. But there is little to suggest that such an evolutionary sequence would be synchronous on neighbouring islands.

With the recent establishment of the Australian Institute of Marine Science at Townsville, a new phase of intensive long term study of ecological and sedimentary processes has become possible. This should go a long way towards rectifying our present ignorance of scale and rate factors operating in the overall barrier system.

In order to capitalise on the geophysical work of the expedition a series of boreholes at intervals across the shelf is needed to demonstrate the nature and sequence of the suspected major Holocene fluviatile development overlying the -109-m terrace: until this is done the interpretation of seismic evidence must remain conjectural. Such a study would be well complemented by detailed examination of the aggradational sequences along the Queensland coast.

On a more local scale the expedition has demonstrated that the time is ripe for 'taking an island apart' by systematically drilling a series of holes through a single island like Bewick, with a view to reconstructing in three, rather than in two, dimensions the evolution of the modern reef geometry. Again, the seismic survey has suggested the potential rewards that such a drilling programme might bring in revealing exactly how the morphology of the earlier dissected reef limestone surface influences the subsequent growth and development of the modern reef system.

Over the next few years, therefore, we should see a new understanding emerge of the internal structure of the Great Barrier Reef, even perhaps including an appreciation of its foundation structure in relation to the plate-tectonic history of the western Pacific.

But there is surely another side to all this. Plate tectonics is all very well, but have we not forgotten the organisms—the very substance and most singular feature of the reefs? Have we yet begun to appreciate, for example, the immense potentiality that the 1,600 km length of reef offers for latitudinal studies of organic diversity? As it is, the basic fieldwork still remains to be done, to enable the spectrum of reef communities of the northern Barrier to be compared with those at Low Isles, let alone with other Indo-Pacific reefs. So we can confidently look forward to a resurgence of interest in the natural history of the Great Barrier, and the despatch of a further, faunistically orientated expedition. □

... and ecology

Reef biogenesis

from Jean H. Weber

A symposium on coral reef biogenesis was organised by the Australian Institute of Marine Science (AIMS) on December 15–16, 1975 in Townsville, Queensland, Australia. The long term objective of the AIMS work on coral reef biogenesis is to develop a unifying model of the growth, development, and maintenance of the Great Barrier Reef, and the symposium was held to identify critical areas of research. Further details may be obtained from the Director, AIMS, Townsville, Queensland 4810.

M. LITTLER (University of California, Irvine) reviewed and evaluated the literature pertinent to the calcium-depositing habit in macroalgae, noting the current debate on the relative ecological importance of various kinds of calcifying organisms. Suggested research areas included partitioning pH changes into those due to calcification and those due to photosynthesis, by following changes in total alkalinity of the system by chemical methods. Studies of periodicity and stress effects on deposition rates were also recommended, as well as of grazing pressure on reef-building algae, inter and intra-specific competition, recruitment, and bioenergetics.

One vital group of reef algae are the symbiotic zooxanthellae, which make possible the survival of reef corals in a nutrient-poor environment. L. Muscatine (University of California, Los Angeles) evaluated the state of knowledge of the coral/zooxanthellar symbiosis. Some fundamental questions still remain unresolved (what, for example, is the quantitative contribution of zooxanthellae to maintenance respiration of a coral? what is the mechanism of nutrient retention and recycling? how does photosynthesis accelerate calcium deposition?).

Marine productivity begins with photosynthesis and the production of metabolic intermediates. A. Benson (Scripps Institution, San Diego) noted that the importance of lipids in the energy budget of a wide variety of reef organisms was first suggested by the recognition of wax esters, triglycerides, and phospholipids as components of mucus secreted by corals and other reef organisms. This mucus is consumed by various animals, including fishes and many commensals. Reef corals derive their stores of lipid by a yet undefined transfer process from their symbiotic zooxanthellae.

M. Doty (University of Hawaii) reviewed the essential functions of the serially appearing algal components of coral reefs: the planktonic (micro-), fleshy (meso-), and crustose (macro-morphic) constituents.

Coral reefs are unique among marine ecosystems in their ability to produce biogenic sedimentary materials and to retain these materials in the form of a wave-resistant structure. This ability to alter the environment is a function both of the rate of production and the nature of the material produced. The growth of reefs is mediated initially by an environmental "coarse-tuning", imposed by geological, meteorological and oceanographic variables. The reef system itself then acts as a "fine-tuning" device. S. Smith (Hawaii Institute of Marine Biology) examined the nature of CaCO₃ materials produced, rates of production, and the external factors which influence and are in turn influenced by the growth of coral reefs.

D. Stoddart (University of Cam-



A hundred years ago

MOST of the natives who come into intimate contact with the Russians at the present time, we are informed, profess Christianity. That many heathen customs still, however, cleave to them is shown by the following incident. At a "simovie" where we landed for some hours on Sept. 16, we as usual came upon a burying-place in the wood near the dwelling-houses. The corpses were laid in large coffins above ground, with a cross in nearly every case raised over them. At one of the graves there was a consecrated picture fixed to the cross, which must be considered an additional proof that a Christian reposed in the coffin. Notwithstanding this, several garments, which had belonged to the deceased, were found hanging on a bush near the grave, together with a bundle containing food, principally dried fish. At the graves of the richer natives we are informed that the survivors place, together with food, some rouble notes, in order that the departed may not be altogether destitute of ready money on his entrance into the other world. But that fine clothes are not considered any special recommendation with St. Peter was evidenced by the exceedingly shabby, tattered, and patched condition of the garments hung up at the grave in question.

from *Nature*, 13, February 17, 313; 1876.

bridge) outlined the geological aspects of reef biogenesis. External form has been used to infer both history and internal structure, without itself being precisely defined. To understand variation in time and space, reefs must be considered as volumes or three-dimensional shapes, as opposed to the commonly-used profile diagram, which is well-adapted to describe zonation but unable to demonstrate spatial variability. New advances in describing reef form have included the use of nearest-neighbour techniques for studying patch-reef distribution, the use of spectral analysis of bottom roughness to describe groove-and-spur formations, the application of the hypsometric integral to describe lagoon basins, and the development of various indices of topographic diversity to describe reef communities. Such quantitative morphometric techniques can be applied on different scales, from entire reef complexes to individual coralla. Until three-dimensional reef forms have been described more precisely in this way, the heavy theoretical loading given to highly simplified reef descriptions must be misleading. □

Spatial contrast vision

from a Correspondent

A Workshop on Spatial Contrast Vision was held in Amsterdam on 9–14 January 1975. It was sponsored by the Commission for Biophysics and Biochemistry of the Netherlands Royal Society of Arts and Sciences.

VISUAL scientists tend to be divided into two camps on the question of how patterns are perceived. One view is that visual information is processed by one or more frequency-selective channels; a strong statement of this theory is that the visual system performs a two-dimensional Fourier analysis. The opposing camp holds that the fundamental building-blocks are the responses of single neurones, the receptive fields of which are sensitive only to specific features of the stimulus; the strong statement is that more central neurones are increasingly selective, and that an individual percept depends ultimately on activity in a single high-order neurone.

This dichotomy could be found in much of the discussion at the Amsterdam meeting. L. H. Van der Tweel (University of Amsterdam) criticised misapplications of Fourier theory: in particular, the tendency to

ignore the question of how phase information is preserved in vision. It was later shown by Ü. Tulunay-Keesey (University of Wisconsin) that phase information can be lost by frequency-selective channels, because equal adaptive effects are found when adapting and test gratings are present in phase or out of phase. This finding raises the question of how spatial channels can ever specify the position of an object.

J. Nachmias (University of Pennsylvania) considered whether vision could be mediated by a single channel, the sensitivity of which varied with the spatial frequency of the stimulus. He concluded that such a model cannot account for known data; a multiple-channel theory is needed. This problem was discussed further by J. G. Robson (University of Cambridge), who described problems in the interpretation of human psychophysical data in terms of the properties of neural receptive fields. The classic concept of a receptive field is of a centre, within which all like stimuli elicit similar responses, and a surround, within which the same stimuli have an opposing effect. New evidence that this is an oversimplification was presented by P. O. Bishop (Australian National University), who described local differences of function within receptive fields. Sensitivity to the direction of stimulus movement can be found in small areas of the receptive field, and cells that exhibit directional sensitivity within the centres of their fields often have non-selective surrounds.

The convention of classifying retinal ganglion cells as X and Y cells was proposed by Enroth-Cugell and Robson in 1966, on the basis that X cells perform a linear summation of light within their receptive fields. The distinction has proved to be useful: neurones with X and Y characteristics have now been found in other parts of the visual system, and they differ in several characteristics. C. Enroth-Cugell (Northwestern University) reported that X and Y cells have different profiles of spatial sensitivity, and may have different neurochemical transmitters. R. M. Shapley (Rockefeller University) showed that only X cells respond linearly to temporal modulation of light. The central projections of X and Y cells differ, as shown by K.-P. Hoffman (Gutenberg University, Mainz). The X cells project only to area 17 of the visual cortex, whereas Y cells project to areas 17 and 18. Hoffman also described a third major category of visual neurones, the W cells, whose only unifying characteristic is their slow conduction velocity.

The ability of many neurones to respond to a limited range of spatial

frequencies was examined by R. L. DeValois (University of California) and L. Maffei (Laboratorio di Neurofisiologia, Pisa). In general, tuning curves become more sharply peaked as one ascends in the visual system. Considerable interest (and perhaps some scepticism) greeted Maffei's conclusion that all neurones that are located in the same column of visual cortex (that is, in a line perpendicular to the surface) tend to respond most vigorously to gratings that have the same orientation, but their preferred spatial frequency changes in a regular pattern as the recording electrode is advanced. Conversely, cells in hypercolumns (tangential to the surface) have different preferred orientations, but all respond to the same spatial frequencies.

Most of these findings emphasise spatial interactions of an inhibitory nature. A. Fiorentini (Laboratorio di Neurofisiologia, Pisa), however presented compelling data that both facilitatory and inhibitory interactions can be found in human vision.

During a 'critical period' in early life, the visual system of many animals is labile and its function can be modified substantially by depriving or distorting the visual input. This phenomenon has been studied extensively because of its implications in the treatment of human amblyopia. C. B. Blakemore (University of Cambridge) described recent experiments in which occlusion of one eye resulted in a selective loss of afferent fibres to the visual cortex from those parts of the lateral geniculate nucleus to which the occluded eye projects. J. Atkinson and O. J. Braddick (University of Cambridge) reported that the contrast vision of human infants can be tested as early as 1 month, and that a rapid improvement in sensitivity occurs during the second month of life. This raised the question of the age at which the critical period begins in man. Bishop stated that the literature suggests 5 months, but Atkinson and R. A. Crone (University of Amsterdam) argued for an earlier onset.

A wide gap still exists between experimental data and an understanding of visual perception. This was emphasised by E. H. Land (Polaroid Corporation), who gave a dramatic demonstration that knowledge of the properties of a discrete physical stimulus is not sufficient to predict its appearance, and by N. S. Sutherland (University of Sussex), who attacked the notion that the central nervous system analyses visual information in a passive manner. In so doing, Sutherland seems to have questioned the meaningfulness of the two popular models of contrast vision that are stated at the beginning of this report.

articles

Hazards of plutonium with special reference to the skeleton

F. W. Spiers

Bone Dosimetry Research, Medical Physics Unit, Cookridge Hospital, Leeds LS16 6QB, UK

Janet Vaughan

Bone Research Laboratory, Nuffield Orthopaedic Centre, Oxford, UK

In the past attempts have been made to deduce plutonium toxicity in man from studies based on animal experimentation. An alternative method is to use the comparative dosimetry of plutonium and radium in man, with results broadly in agreement with maximum permissible levels set by the International Commission on Radiological Protection.

SEABORG recognised as early as 1944 that "the physiological hazards of working with plutonium and its compounds may be very great"¹. Subsequent experimental work on the biological behaviour of plutonium has confirmed Seaborg's fear about his new element and has shown that it is more carcinogenic than radium. As increasing quantities of plutonium will be produced in connection with nuclear energy programmes, it is important to have some estimate of the risks involved, namely the possible incidence of malignant cancers in individuals who have acquired a plutonium burden in the course of their work.

We present here some estimates of the risk of development of malignancies associated with the skeleton, based on a dosimetric comparison with the incidence of radium-induced cancer in man, about which considerable evidence exists^{2,3}. Both ²²⁶Ra and ²³⁹Pu emit α particles with not very different energies and both are deposited in bone; ²²⁶Ra is deposited throughout the volume of mineral bone whereas ²³⁹Pu is deposited on bone surfaces, with some distributed in bone marrow. Our calculations, however, take these differences into account since they are based, as UNSCEAR⁴ recommends, on the known cells at risk and the determination of the physical dose these cells receive. Similar dose calculations cannot yet be made for the other major sites of plutonium retention, the lung and the liver, because the cells at risk are not known with the same certainty.

Cells at risk in the skeleton

The cells at risk in the case of the skeleton are the progenitor osteogenic cells on bone surfaces, all the progenitor cell types found in the marrow and cells in the epithelium closely applied to bone in the sinuses of the skull^{5,6}. The following brief review illustrates the evidence available that malignancies have been found in all these tissues, in animals and man, when irradiated by α particles. Of the tumours listed, osteosarcomas are by far the most numerous.

The data on ²³⁹Pu refer to experimental animals with known

plutonium burdens. In 75 dogs injected with ²³⁹Pu (ref. 7), 55 osteogenic sarcomas were recorded, compared with one case of leukaemia. There were no osteosarcomas in the controls but one leukaemia occurred.

In rats studied by Benstead *et al.*⁸ 45 osteosarcoma were recorded against four cases of myeloid leukaemia. Russian workers⁹ comment on a high incidence of tumours of the blood-forming organs in rats but data on osteosarcoma are not given. In extremely careful studies in mice, Loutit (personal communication) has recorded generalised lymphomas and local lymphomas as well as osteosarcomas and angiosarcomas. The mouse studies of Finkel and Biskis¹⁰ show a high incidence of both osteosarcomas and reticular tumours, but are difficult to assess because of the large number of reticular tumours in the controls. One carcinoma of the sinuses is recorded by Jee⁷ in a beagle dog whereas none were seen in controls.

No malignancies attributable to plutonium have been recorded in man, although in some cases the body burdens have been as high as 0.42 μ Ci (ref. 11). Tumours have occurred however, in a large group of persons (1,346 in all) exposed to radium, as either ²²⁶Ra or ²²⁸Ra or a mixture of the two. For this population information is available on radium body burden, calculated radiation dose received and subsequent pathology^{2,3,12,13}. The total number of osteosarcomas in the radium study when last reported was 51; there were 22 cancers of the sinuses^{3,13}.

Some patients had both types of tumour. Finkel and his colleagues² include in their record two cases of myeloid leukaemia and three other less well defined blood dyscrasias. When these figures were taken from the recent analysis of the radium cases, some of the early patients described by Martland¹⁴ were not included by Rowland *et al.*³. In addition to osteogenic sarcoma, these early cases had atypical dysplasia of the marrow associated with severe anaemia. Their body burdens of radium were extremely high. Loutit¹⁵ has suggested that they should be regarded as samples of malignant transformation of marrow cells. The risk of leukaemia from a plutonium burden is possibly greater than that from a radium burden as plutonium is retained in marrow as well as in the bone itself¹⁶⁻²¹. An ICRP publication²² therefore suggested in 1972 that the skeleton probably should be considered as two distinct organs, one comprising the cortical and trabecular bone and the other the bone marrow. It may be noted that the time taken for leukaemia to develop is likely to be longer than in the case of external radiation since plutonium, entering the body by inhalation or tissue wounding, will translocate slowly and continuously from lungs, lymph nodes, wound site or initial liver deposits. The marrow dose will build up slowly with time²¹.

Animal work and toxicity estimates

Attempts have been made to relate the toxicity of a known body burden in dogs injected with different radionuclides to known body burdens of radium in man using the formula

$$\frac{\text{dose from X in animal}}{\text{dose from } ^{226}\text{Ra in animal}} = \frac{\text{dose from X in man}}{\text{dose from } ^{226}\text{Ra in man}}$$

where the doses refer to some given level of toxicity.

The doses for dogs and humans are based on an average distribution of both radionuclides in bone, that is on the average dose to mineral bone. The UNSCEAR report²³ makes a general comment on such types of comparison between radium data for man and animal data: "this question carries the assumption that the ratio of the absorbed doses which produce different effects for different radionuclides will be the same in man and the experimental animal, independent of promoting host factors which might act differentially, an assumption that at present remains unproved". The ratio of the doses to the cells at risk for ²³⁹Pu and ²²⁶Ra are not necessarily the same as the ratio of the absorbed doses to bone. Apart from the fact, pointed out by UNSCEAR²³, that it is unsatisfactory to base toxicity estimates on average dose to mineral bone when it is dose to sensitive tissues that is important, there are other difficulties involved in this comparison of data for dogs and humans. First, Spiers²⁴ has shown by direct measurement of both dog and human bone that considerable structural differences in the two species affect the dose to the sensitive tissues in marrow and on bone surface and are not taken into account by the average dose to mineral bone.

Table 1 Calculated dose rates

Radionuclide	Mean dose rates (rad yr ⁻¹) for 1 µCi skeletal content*			
	Trabecular marrow	Trabecular endosteum	Cortical endosteum	Air-sinus epithelium
²²⁶ Ra + retained daughter elements	1.6 (1.5)†	19 (18)†	35	275‡
²³⁹ Pu	16.9	129	193	193‡

* Bone data assumed: skeletal mass = 5 kg, total endosteal area = 16 m².

† Deduced from data (plane slab calculation) by Charlton and Cormack³¹.

‡ Estimate only.

Second, since plutonium is a surface seeker, the rate at which resorption and apposition occurs will affect the dose received by sensitive tissues. There is no reason to suppose that 'turnover rate' is the same in man and dog and it will certainly not be the same in young dogs and adult man as is accepted in the above formula. Marshall and Lloyd²⁵ have indeed drawn attention to the fact that it is unwise to compare toxicity in young dogs with that in adult man since the rate of bone turnover, that is both removal of plutonium-containing bone and its burial by new bone formation, may be different. As a result of their calculations of different remodelling times in young dogs and adult man, Marshall and Lloyd conclude that in the case of monomeric plutonium the relative biological effectiveness of plutonium compared with radium (RBE) in man is about three times that in dogs.

The differences between the skeletons of young dogs and adult humans are again emphasised by a study²⁶ of the relative carcinogenicity of ²²⁶Ra and ²²⁸Ra in man and a comparison with the results for the Utah dogs⁷. In the case of the Utah dogs, ²²⁸Ra was more carcinogenic than ²²⁶Ra with an RBE of 2.5. Rowland *et al.*²⁶ concluded, however, that in man there was no difference in the toxicity of the two isotopes. As they say, these observations make it difficult to accept that toxicity ratios observed in beagles can be used directly to predict toxicity ratios

in man. Although examination of the data they give for cumulative dose levels above 1,000 rad does not altogether substantiate their claim that there is no statistical difference between the toxicity of ²²⁸Ra and ²²⁶Ra in man, it seems probable that the comparison of the data for dogs and humans gives too high an RBE for ²²⁸Ra relative to ²²⁶Ra in humans. On theoretical grounds ICRP²⁷ has put the maximum permissible body burden (MPBB) for ²²⁸Ra lower than that for ²²⁶Ra.

Since considerable reservations must be made in interpreting simple translation to man of toxicity data obtained with dogs, it is worthwhile attempting to estimate the toxicity of ²³⁹Pu by a method that uses the data for ²²⁶Ra in man coupled with new calculation of dose to the relevant tissues.

Dose rates calculated from skeletally deposited radionuclides

A method of calculating the radiation dose to tissues in bone from skeletally deposited radionuclides has been devised that takes into account the physical dimensions of the trabeculae and marrow spaces in trabecular bone^{24, 28, 29}. It is based on the principle that particles cross the small marrow cavities in approximately straight lines with varying lengths of path. A complete description of all possible path lengths through the marrow spaces and through the trabeculae makes it possible to calculate the fraction of energy deposited, and hence the dose, in the marrow spaces by particles arising in the trabeculae. This is done by a Monte Carlo method operating in probability distributions of random path lengths measured by a specially-designed bone-scanning microscope³⁰. The dose is averaged over the whole distribution of path lengths to give a mean value for a given bone, and sufficient bone specimens are analysed for a mean dose for the whole skeleton to be obtained. Mean dose rates have been calculated for a skeletal content of 1 µCi of a given radionuclide for the following tissues: (1) bone marrow in trabecular bone (*D_M*); (2) endosteal tissue, 10 µm thick, on trabecular bone surfaces (*D_s*); (3) endosteal tissue on the surfaces of cavities in cortical bone (*D_c*), and (4) epithelial tissue adjacent to bone in the air sinuses (*D_{AS}*).

Table 2 Calculated risks

Tissue	Late effect	Risk coefficient: cases per 10 ³ per yr for 1 rad yr ⁻¹
Trabecular marrow	Leukaemia	<i>r_M</i> = 0.101
Trabecular endosteal surfaces	Osteosarcoma	<i>r_{OS}</i> = 0.079
Cortical endosteal surfaces	Osteosarcoma	<i>r'_{OS}</i> = 0.016
Air-sinus epithelium	Carcinoma	<i>r_{AS}</i> = 0.003

The method of calculation, developed initially for β emitters, assumes that the radionuclide is deposited uniformly throughout the volume of the mineral bone. It can be applied equally to a "volume-seeking" α emitter such as ²²⁶Ra and results for this radionuclide are given in Table 1. Where comparisons can be made, the dose rates agree well with those derived from data by Charlton and Cormack³¹ for the dose near a plane slab of bone containing ²²⁶Ra (Table 1).

In the case of those radionuclides, such as ²³⁹Pu, that are deposited on bone surfaces the Monte Carlo program must be modified to consider particles as originating only at the endosteal points of any given trabecular path. A further factor must be introduced to allow for the contribution to the dose from the trabecular surface immediately adjacent to the cavity concerned. The details of the calculation for "surface-seekers" will be published later, but approximate dose rates for a skeletal content of 1 µCi ²³⁹Pu are given in Table 1. For the purpose of this article it has been assumed that 10% of the skeletal content could be deposited in bone marrow²¹, with the remainder distributed uniformly over the endosteal surfaces of the

skeleton. The dose rates calculated for ^{239}Pu agree reasonably well with those based on a thin plane source. Thus the dose rate for the trabecular endosteum is about 10% less than that calculated for a thin plane³². It is about 25% less than the value deduced from the data of Marshall *et al.*³³ but in this case uniform energy deposition along the particle track was assumed. Dose rates near trabecular surfaces may be expected to be somewhat lower than for a thin plane source because the trabecular surfaces are not flat and the marrow spaces are generally too large for appreciable cross contributions to the dose from other trabecular surfaces. No allowance has been made for possible burial of the Pu by bone growth because insufficient evidence is available; the dose rates in Table 1 are therefore likely to be maximum estimates. In the case of ^{238}Pu , the α -particle energy and the consequent dosimetry are almost the same as for ^{239}Pu , though it must be remembered that because of its high specific activity ^{238}Pu does not necessarily behave biologically like ^{239}Pu .

Calculation of total risk to bone

The principle of the calculation³⁴ depends on a knowledge of the risk coefficients for the relevant late effects (leukaemia, bone sarcoma and air-sinus carcinoma) when bone tissues are irradiated by α particles. The coefficients are expressed as the equilibrium risk rates, namely, the number of cases per 1,000 persons per year when a dose rate of 1 rad yr⁻¹ is maintained for a sufficiently long time. The 'equilibration' time depends on the latency period of tumour appearance, and for irradiated bone this is likely to be long—bone tumours have occurred from 10 to 30 yr after irradiation.

The total risk rate, R_1 , for a skeletal content of 1 μCi of a given radionuclide can be stated as

$$R_1 = r_M D_M + r_{OS} D_S + r'_{OS} D'_S + r_{AS} D_{AS} \quad (1)$$

where r_M is the risk coefficient for leukaemia per unit dose rate and D_M is the dose rate to bone marrow per μCi skeletal content; $r_{OS} D_S$ refers similarly to bone sarcoma in endosteal tissues in trabecular bone, $r'_{OS} D'_S$ to bone sarcoma in cortical bone and $r_{AS} D_{AS}$ to air-sinus carcinoma. Clearly other risks, if any, could be added but these particular ones will be considered in respect of the α irradiation of bone.

and the total risk rates to bone are as given in Table 3 for ^{226}Ra and ^{239}Pu . The risk estimates for plutonium are made on the almost axiomatic assumption that the effect of unit α -particle dose, delivered under the same conditions to the target cells concerned, does not depend on the radionuclide from which the α particles originate (apart from possible minor changes in RBE with α -particle energy).

The data in Table 3 can be used to derive a quantity, $q(\mu\text{Ci})$, which we can define as the skeletal content which results in some stated maximum risk rate R_{MPL} ; the value of q is then obtained from the relationship

$$q R_1 = R_{MPL} \quad (2)$$

If bone only is irradiated by the bone-seeking radionuclide, the value of R_{MPL} could be taken, for example, as the risk rate associated with the limit 5 rad yr⁻¹, the maximum permissible dose rate set by ICRP for radiation workers. This value can be deduced from the conclusions of the UNSCEAR report³⁶ as about 0.5 cases per 1,000 per yr, and q is then given by

$$q = 0.5/R_1 \quad (3)$$

Values of q are listed in the penultimate column of Table 3 and the present ICRP values of the MPBB²⁷ in the last column. It may be noted that, in deriving q , linear dose-response relationships have been assumed on both sides of equation (2), also that the occurrence of air-sinus carcinoma in the radium series is almost certainly due to heavy α irradiation resulting from trapped radon in the poorly ventilated sinus cavities, which does not happen with plutonium.

The ICRP figures are based on the MPBB of 0.1 μCi assigned to ^{226}Ra , a value that has remained unchanged for some 30 yr. The limit 0.1 μCi was set by the clinical evidence of bone cancer that had occurred by the early 1940s in the comparatively large group of persons who had acquired a body content of radium. The values of the MPBB for other α emitters are set by the effective energy deposited in the skeleton compared with that deposited by 0.1 μCi ^{226}Ra and the daughter elements in the ^{226}Ra decay chain²⁷. The ICRP values in Table 3 are based on a deposition of 99% of the body content of ^{226}Ra and 90% of ^{239}Pu in the skeleton²⁷. Some comparison can therefore be

Table 3 Risk rates

Radionuclide	Risk rate: equilibrium no. cases per 10 ³ per yr for maintained skeletal content of 1 μCi				Total	q (μCi)	MPBB (ICRP) (μCi)
	Leukaemia	Bone sarcoma		Carcinoma			
		Trabecular	Cortical				
^{226}Ra	0.2	1.5	0.6	0.8	3.1	0.16	0.1
^{239}Pu	1.7	10.2	3.1	0.6	15.6	0.032	0.04

The values of the risk coefficients can be deduced from the data for the radium cases. In 777 cases reported by Rowland *et al.*³ the mean dose to bone was 1,860 rad and the malignancies observed over 40 yr were: 51 cases of osteosarcoma and 20 cases of air-sinus carcinoma. In another analysis by Finkel *et al.*² covering many of the same cases there were several blood dyscrasias of which at least three were acceptable as myeloid leukaemias (case histories and histology seen by J. V.). For our purpose of risk calculation we have taken the perhaps arbitrary figures of four cases of leukaemia. Details of the derivation of the risk estimates for α particles will be given elsewhere. The calculations are on a linear dose-response basis and allow for the power-law reduction of dose rate with time after acquisition of the radium³⁵. The results are given in Table 2, as the equilibrium numbers of cases per 1,000 per yr for a maintained dose rate of 1 rad yr⁻¹.

If the dose rates in Table 1 are multiplied by the corresponding risk coefficients, the risk rates to the various tissues in bone

made between the skeletal contents, q , and the MPBB values. The comparison can only be approximate because the ICRP recommendations²⁷ require that the MPBB for long-lived radionuclides, such as ^{226}Ra and ^{239}Pu , shall only be reached after 50 yr occupational exposure. In our calculations the values of q are the skeletal contents which, if maintained, will result in the stated risk rate by the end of an equilibration time of some 30 yr or more. Nevertheless, the general concordance between two very different approaches to determining a maximum permissible skeletal content is such that the MPBB for ^{226}Ra still seems valid and that for ^{239}Pu is not in need of major revision in respect of bone. If, however, more recent ICRP data²² on the distribution of ^{239}Pu in the body are considered (45% in bone and 45% in liver), and the liver risk can be assessed adequately, some revision of the MPBB for ^{239}Pu may be necessary.

The data in Table 3 can also be used to calculate the expected number of cases if a skeletal burden of 0.04 μCi ^{239}Pu were

maintained for about 50 yr; the expectation would be about two cases of leukaemia and 13 cases of bone sarcoma per 1,000 persons so exposed. Such estimates are necessarily tentative in view of the assumptions involved; they may be too high because the dose-response relationship so far reported for ^{226}Ra is non-linear and a more detailed analysis (as that by Rowland *et al.*³), might lead to much lower estimates.

Implications and conclusions

Because, as we have shown, it is difficult to translate animal toxicity data directly to man, there is an advantage in estimating plutonium toxicity on α -particle dosimetry in human bone and data on cancer induction in man by ^{226}Ra . This has involved some simplifications of the very complex radiobiological problems involved. For example, we have followed the ICRP and UNSCEAR in assuming a linear dose-response relationship. Other relationships are possible and a detailed theoretical study of the consequences of adopting different dose-response formulae has been made by Mayneord and Clarke³⁷. Any formula adopted would have to be applied to both sides of equation (2). Further, we have assumed uniform volume deposition of ^{226}Ra and uniform surface deposition of ^{239}Pu in the skeleton. If a linear dose-response relationship holds then non-uniformity does not affect the expectation of cancer induction; there is indeed some evidence³⁸ from the induction of bone sarcoma in mice by ^{45}Ca , ^{90}Sr and ^{226}Ra that tumour numbers follow the average bone dose rather than the dose in the "hot spots". It should also be noted that in the case of inhaled plutonium, and with wound contamination, the plutonium at the local site will change with time and generally the bone content and the bone dose rate will increase with time. Also the risk to cells in the marrow will arise in part from plutonium that has translocated from elsewhere and this, as in the case of Thorotrast, takes time to reach an effective level^{39,40}.

In making our analysis we have not considered the 'hot particle' problem which is as yet incompletely resolved and which is especially important in relation to cancer risk in the lung⁴¹⁻⁴⁴. We have some comments to make, however, which seem to be relevant in respect of the risk when only a few cells in any one organ are irradiated. Recently, Mayneord and Clarke have extended their studies of point sources of β particles³⁶ to the case of point sources of ^{239}Pu (ref. 45), and found that a peak probability of malignant change, 1.5×10^{-10} per hot particle, occurs at an integrated particle activity of $0.3 \mu\text{Ci s}$. We find we can deduce a risk of the same order of magnitude from data on the occurrence of osteosarcoma in the long bones of the radium cases. Measurements of trabecular surface areas by Spiers *et al.* (to be published later) have shown that the incidence of osteosarcoma in segments of the long bones is proportional to trabecular surface area with an incidence over a period of some 40-50 yr, of 2.4×10^{-6} per cm^2 of trabecular surface. Because the osteosarcoma cases represent various radium burdens, this correlation suggests a worst-case situation in which, over long periods of exposure, the endosteal surface doses approach a carcinogenic level and the outcome is proportional to the area, that is, to the number of endosteal cells put at risk. If we were to make the admittedly large extrapolation to a shell of cells surrounding a ^{239}Pu hot particle of radius equal to the α -particle range (35 μm in tissue) we would get a risk of malignant change for the area concerned ($1.6 \times 10^{-4} \text{ cm}^2$) of about 4×10^{-10} per particle.

If we accept the risk estimate of Mayneord and Clarke of 1.5×10^{-10} per particle and take an average lung residence time of 2 yr for insoluble particles, the particle activity for peak probability is $0.5 \times 10^{-2} \text{ pCi}$ and the number of hot particles for one maximum permissible lung burden (MPLB) of 16,000 pCi is 3.2×10^6 . The expectation of malignant change for one MPLB would then be 5×10^{-4} spread over a long period of years. If one MPLB were received annually over 50 yr the total expectation of lung cancer would be about 12 cases per 1,000, not very different from the total risk to bone and bone marrow given earlier in this article. Reservations must be made in regard

to all such calculations because no account can be taken at present of the effects of particle movement.

Summary

Since our calculations of "maximum risk" skeletal contents for the radionuclides ^{226}Ra and ^{239}Pu accord well with values of the MPBBs arrived at by the ICRP in 1959 on other grounds, we conclude that the MPBB of $0.04 \mu\text{Ci}$ for ^{239}Pu , then agreed, is not in need of major revision in respect of bone. A maintained skeletal burden of ^{239}Pu of $0.04 \mu\text{Ci}$ would lead, on our calculations, to possibly two cases of leukaemia and 13 cases of bone sarcoma per 1,000 persons over about 50 yr.

Carcinoma of the air sinuses would present a very much smaller risk than bone cancer. The risk to the lung cannot be so readily evaluated because, among other uncertainties, the cells at risk and the possible movement of the sources of radiation are little understood.

The fact, however, that recent studies by Mayneord and Clarke lead to risk estimates for the lung that are of the same order as those we find for bone suggests to us that, as far as we know, the maximum permissible lung burden set by the ICRP can also reasonably be accepted.

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- Seaborg, G. T., in *Radiobiology of Plutonium* (edit. by Stover, B. J., and Jee, W. S. S.), 1-21 (J. W. Press, Salt Lake City, 1972).
- Finkel, A. J., Miller, C. E., and Hasterlik, R. J., in *Delayed Effects of Bone Seeking Radionuclides* (edit. by Mays, C. W., *et al.*), 195-225 (University of Utah Press, Salt Lake City, 1969).
- Rowland, R. E., Failla, P. M., Keane, A. T., and Stehney, A. F., in *Argonne natn. Lab. Rep. ANL 7760*, Part II, 1-17 (1969-70); *ibid* 7860, Part II, 1-8 (1970-71).
- Rep. UN Scientific Committee on the Effects of Atomic Radiation, 2, 392 (United Nations, New York, 1972).
- International Commission on Radiological Protection, in *A Review of the Radiosensitivity of the Tissues in Bone*, Publ. No. 11 (Pergamon, Oxford, 1968).
- Loutit, J. F., and Vaughan, J., *Br. J. Radiol.*, 44, 815 (1971).
- Jee, W. S. S., in *Research in Radiobiology*, COO 119, 249, 24-39 (1974).
- Bensted, J. P. M., Taylor, D. M., and Sowby, F. D., *Br. J. Radiol.*, 38, 920-925 (1965).
- Moskalev, Y. I., Stretsova, V. N., and Buldakov, L. A., in *Delayed Effects of Bone Seeking Radionuclides* (edit. by Mays, C. W., *et al.*), 489-509 (University of Utah Press, Salt Lake City, 1969).
- Finkel, M. P., and Biskis, B. O., *Health Phys.*, 8, 565-579 (1962).
- Hempelmann, L. H., Richmond, C. R., and Voelz, G. L., *Los Alamos Sci. Lab. Rep. LA 5148 MS*, Informal Rep. UC 41 and 48 (1973).
- BEIR Rep., 128 (National Academy of Sciences, National Research Council, Washington, DC, 1972).
- Argonne natn. Lab. A. Rep., ANL 8060, 289-333 (1972-73).
- Martland, H. S., *Am. J. Cancer*, 15, 2435-2516 (1931).
- Loutit, J. F., *Br. J. Cancer*, 24, 195-207 (1970).
- Langham, W. H., Bassett, S. H., Harris, P. S., and Carter, R. E., *Los Alamos Sci. Lab. Rep. LA 1151* (1950).
- Morrow, P. E., *et al.*, *Health Phys.*, 13, 113-133 (1967).
- Vaughan, J., Bleaney, B., and Williamson, M., *Br. J. Haem.*, 13, 492-502 (1967).
- Rosenthal, M. W., and Lindenbaum, A., in *Delayed Effects of Bone Seeking Radionuclides* (edit. by Mays, C. W., *et al.*), 489-509 (University of Utah Press, Salt Lake City, 1969).
- Buldakov, L. H., Lyubchanskii, E. R., Moskalev, Y. I., and Nifatov, A. P., *LF-tr-41 UC-48 (Atom Publications, Moscow, 1970)*.
- Bleaney, B., and Vaughan, J., *Br. J. Radiol.*, 44, 67-73 (1971).
- International Commission on Radiological Protection in *Report on Metabolism of Plutonium and Related Elements and Compounds* (Pergamon, Oxford, 1972).
- Rep. UN Scientific Committee on the Effects of Atomic Radiation, 2, 383 (United Nations, New York, 1972).
- Spiers, F. W., *Br. J. Radiol.*, 47, 833-844 (1974).
- Marshall, J. R., and Lloyd, E., in *Radionuclide Carcinogenesis*, AEC Symp. Series 29, Conf. 720505, 421-436 (1973).
- Rowland, R. E., Keane, A. T., and Lucas, H. F., in *Radionuclide Carcinogenesis*, AEC Symp. Series 29, Conf. 720505, 406-420 (1973).
- International Commission on Radiological Protection, in *Rep. Committee II on Permissible Dose for Internal Radiation*, 3 (Pergamon, New York, 1959).
- Whitwell, J. R., and Spiers, F. W., in *Proc. Fifth Cong. French Soc. for Radiation Protection*, Grenoble, 401 (1971).
- Whitwell, J. R., and Spiers, F. W., *Phys. Med. Biol.*, 21, 16-38 (1976).
- Darley, P. J., in *Proc. Symp. Microdosimetry*, EAEC Publication EUR 3747, d-f-e, 509-526 (1968).
- Charlton, D. E., and Cormack, D. V., *Br. J. Radiol.*, 35, 473-477 (1962).
- Thorne, M. C., *Nature*, 259, 539-541 (1976).
- Marshall, J. H., Groer, P. G., and Schlenker, R. A., in *Proc. Alta Conf.*, 1974 (University of Utah Press, in the press).
- Spiers, F. W., in *Proc. Third Int. Cong. Int. Radiat. Protection Ass.*, Washington, 1973, Conf. 330907-PI, 439 (USAEC, Oak Ridge, Tennessee, 1974).
- Norris, W. P., Speckman, T. W., and Gustafson, P. F., *Am. J. Roentgenol.*, 73, 785-802 (1955).
- Rep. UN Scientific Committee on the Effects of Atomic Radiation, 1, 9 and 2, 303-358 (United Nations, New York, 1972).
- Mayneord, W. V., and Clark, R. H., *Br. J. Radiol.*, Suppl. No. 12 (1975).
- Marshall, J. H., and Finkel, M. P., in *The Relation of Radiation Damage to Radiation Dose in Bone*, 40-44 (International Atomic Energy Agency, Vienna, 1960).
- Vaughan, J. M., *The Effects of Irradiation on the Skeleton* (Clarendon Press, Oxford, 1973).
- Abbott, J. D., in *Radionuclide Carcinogenesis*, AEC Symp. Series 29, Conf. 720505, 451-465 (1975).
- MRC Report on the Toxicity of Plutonium (HMSO, London, 1975).
- Rep. R 29 (National Radiological Protection Board, Harwell, 1974).
- Lovins, A. B., and Patterson, W. C., *Nature*, 254, 278-280 (1975).
- Bair, W. J., Richmond, C. R., and Wacholz, B. W., *USAEC Rep. Wash-1320* (US Atomic Energy Commission, Washington DC, 1974).
- Mayneord, W. V., and Clarke, R. H., *Central Electricity Generating Board Report RD/B/N 2878* (1974).

Quantitative assessment of carcinogenic risks associated with 'hot particles'

W. V. Mayneord

Institute of Cancer Research, University of London, UK*

R. H. Clarke

Berkeley Nuclear Laboratories, Berkeley, Gloucestershire GL13 9PB, UK

We have examined the hot particle problem in terms of the number of irradiated cells and the doses to which they are subjected, assuming both linear and nonlinear dose-response functions typical of those reported in the radiobiological literature. We assess the risk associated with point sources of α , β and γ activity compared with uniform irradiation, as well as the effects of fractionation of a single particle, and the change in risk if the particle moves. Finally, although the biological data are insufficient for firm estimates, we have tentatively attributed absolute carcinogenic risks in these various situations.

THE recommendations of the International Commission on Radiological Protection assume that there is no threshold in the biological response to radiation, and that effects increase linearly with dose¹. As a consequence, the average dose to an organ is a measure of carcinogenic risk, and concentration of the same energy into smaller volumes, which thereby locally sustain higher doses, leads theoretically to an identical probability of overall biological effect. The assumption of any form of dose-response relationship other than linear implies that spatial distribution of energy deposition may be important.

A special case of great theoretical and practical importance is the point source of α or β activity, which subjects only a small fraction of the cells of an organ to very large doses. This 'hot particle' problem has become the subject of considerable controversy since Tamplin and Cochran² concluded that the associated tumorigenic risk is many orders of magnitude greater than that estimated from mean organ dose on the hypothesis of linearity. More recently, Lovins and Patterson³ have questioned whether the present protection levels for α emitters are adequate. The biological evidence concerning this alleged increased risk has been reviewed by Bair, Richmond and Wachholz⁴ and also by the UK Medical Research Council⁵, both reports concluding that "there is at present no evidence to suggest that irradiation of the lung by particles of plutonium is likely to be markedly more carcinogenic than when the same activity is uniformly distributed".

Theoretical model

Our fundamental assumption⁶ is that the biological response of a single cell (that is, the probability of a relevant biological effect) is some function $\phi(p_c D)$ of dose D , where p_c is the probability of malignant disease per cell per unit dose. The value of f_c has often been assumed constant, but is, in reality a

function of position, since the probability of effective emergence of a cell after 'transformation' may depend on local physiological and histological conditions, oxygen tension, cell contacts and the like. Indeed p_c is a function of many biological and physical variables, and for a given system may be periodic, varying during the cell cycle. Assuming, however, an overall mean value of p_c , and that the dose is spatially represented by $D = f(x, y, z)$, the overall expectation (ϵ) of malignancy in an organ or tissue may be written

$$\epsilon = \int n \phi\{p_c f(x, y, z)\} dV \quad (1)$$

where n is the number of cells per unit mass of tissue liable to undergo the effect. n will also probably be a complicated function of position and may also be a function of time.

The response, $\phi(p_c D)$, may take a variety of forms, but those of particular interest to us are the experimentally observed carcinogenic dose-response relationships. These often behave as power laws of dose at low doses, rise to a maximum in the region of a few hundred to a few thousand rads, the response then decreasing at higher doses. Many examples of this type of behaviour have been cited by UNSCEAR⁷, Mole⁸ and ourselves⁶. The decrease in biological effect at high doses may arise for a variety of reasons ranging from loss of reproductive capacity⁸ or 'cell death' to simple Poisson probabilities of events⁶. We have often used one form of response of particular importance, namely

$$\phi(p_c D) = p_c^2 D^2 \exp(-\lambda D) \quad (2)$$

as representative of a range of biological observations.

Only for uniform irradiation of an organ or tissue can we expect the observed macroscopic dose-response relationship to have the same form as the cellular dose-response function⁶.

Point source β and γ emitters

We have integrated equation (1) using the cellular response function (2) for β -emitting single point sources of high and low energy⁶. The high energy source chosen was ⁸⁶Rb (91.2%, 1.78 MeV β ; 8.8%, 0.71 MeV β), the small γ contribution being ignored. The low energy source was ³⁵S (100%, 0.167 MeV β). For both sources, the dose-distance data tabulated by Cross⁹ were used. The values of λ chosen (5×10^{-4} and 5×10^{-3} rad⁻¹) correspond to peaks in the dose-response function (2) at 4,000 and 400 rad respectively. The results of integration, performed numerically by computer, are shown in Fig. 1 as a function of the integrated source activity (Ci s).

When the same amount of energy is uniformly distributed throughout mass M , the corresponding expectation ϵ_u for the same dose-response function (2) is

$$\epsilon_u = M n p_c^2 \bar{D}^2 \exp(-\lambda \bar{D}) \quad (3)$$

where $\bar{D}$ is the mean organ dose.

*Present address: 7 Downs Way Close, Tadworth, Surrey KT20 5DR, UK.

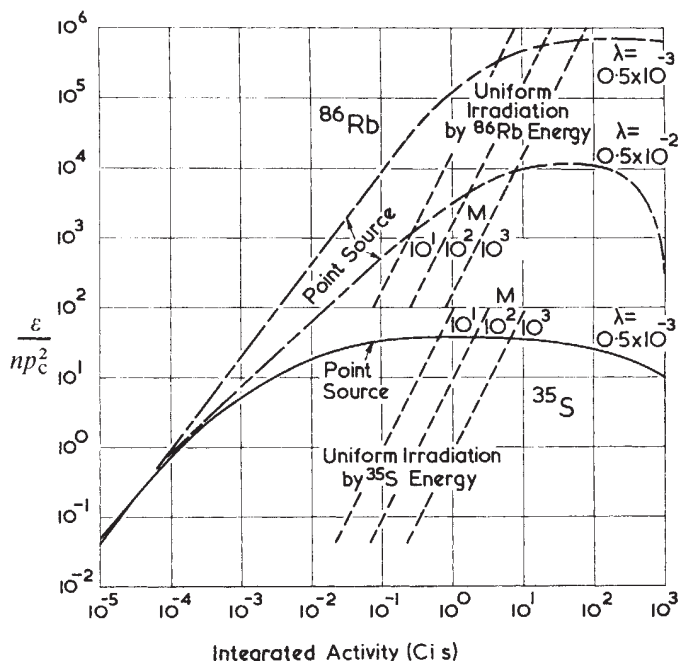


Fig. 1 Expectation of a malignancy from point sources of high (^{86}Rb) and low (^{35}S) energy β emitters, as a function of source activity compared with a uniform distribution of the same amount of energy through different masses $M(\text{g})$. The cellular response function was $\phi(p_c D) = p_c^2 D^2 \exp(-\lambda D)$ for $\lambda = 5 \times 10^{-4}$ and $5 \times 10^{-3} \text{ rad}^{-1}$.

The results of equation (3) are also plotted in Fig. 1, where the ordinate is not the expectation, ϵ , but the related quantity ϵ/np_c^2 . We will return later to the evaluation of np_c^2 required to transform the expectations from equations (1) and (3) to absolute values.

From Fig. 1 we see that for tissue masses of more than $\sim 1 \text{ g}$ and for low source strengths, the point-source expectation exceeds that from uniform irradiation. At high source strengths, the opposite may be true. The mean organ dose at which the crossover occurs increases with the β energy of decay and decreases with increasing organ mass.

The effect of reducing the dose at which the peak in the response function occurs, that is, increasing λ , is to reduce greatly the single point-source expectation. During the rest of this paper, we have concentrated on the dose-response function peaking at 4,000 rad ($\lambda = 5 \times 10^{-4} \text{ rad}^{-1}$) since it predicts the higher expectations.

We note that the ratio of point-source expectation to that for uniform distribution of the same amount of energy is greatest at very low source strengths, that is, for small values of absolute expectation. We see too that the point source expectation rises roughly linearly with source strength. This rather surprising result suggests that the total energy absorbed is still an important parameter.

We have next applied the theory to a mixed β - γ emitter, ^{60}Co , with γ rays of 1.17 and 1.33 MeV per disintegration and β -rays of 0.318 MeV. The resulting expectations are shown in Fig. 2 for a 1-kg sphere of tissue. We see that above 10^{-1} Ci s (0.12 rad), the γ contribution adds significantly to the single point-source expectation, so that uniform irradiation becomes more 'carcinogenic' only beyond 10^2 Ci s (120 rad to 1 kg). We note that both the absolute energy and the ratio of γ to β decay affects the mean dose at which uniform irradiation becomes more carcinogenic.

Point source α emitters

The range of α particles is much smaller than that for most β -radiation, and the resulting doses are, correspondingly,

confined to very localised volumes. The dose-distance function assumed for ^{239}Pu was

$$D = 1.83 \times 10^{-8} \left(\frac{0.69}{r^2} + 7.22 \times 10^4 \right) \text{ rad per disintegration} \quad (4)$$

(J. R. Harvey, personal communication) where r is the distance from a point source, if less than the assumed mean range of the three ^{239}Pu α -particles ($r \leq 35.5 \mu\text{m}$). The results of integrating equation (1) for a single point source of varying strength, using the response function (2) peaking at 400 and 4,000 rad respectively, are shown in Fig. 3, together with the results for uniform distribution of the same amount of energy throughout 1 g of tissue.

Again we see that at very low source strengths there is an increased relative risk from a single point source of ^{239}Pu compared with uniform irradiation. At higher source strengths the expectation falls more steeply than with a β -particle source because of the assumed form of the α dose-distance function. Uniform irradiation becomes more carcinogenic beyond about $3 \times 10^{-6} \text{ Ci s}$ for response functions peaking at 4,000 rad and beyond $3 \times 10^{-7} \text{ Ci s}$ for those peaking at 400 rad. The effect of changes of value of λ in equation (2) are thus even more marked for α sources than β emitters.

From Fig. 3 we see that, for a point source, at $\geq 10^{-8} \text{ Ci s}$ 'cell killing' predominates even for the smaller value of λ . One may question the use of our homogeneous dose function, when individual cells have highly ionising tracks passing through a small fraction of their volume. The detailed pattern of distribution of energy loss from an α particle passing through a single cell may be important⁶, and the stochastic methods of microdosimetry developed by Rossi and Kellerer¹⁰ may be more appropriate. We note, however, that our model suggests a peak expectation at about the same integrated source strength as predicted recently by Bair *et al.*¹¹ on a stochastic basis assuming one α -particle traversal per cell.

We also note that our absolute estimates (below) of risk are of the same order as that deduced by Spiers and Vaughan¹² for the case of small numbers of cells subject to the risk of osteogenic sarcoma.

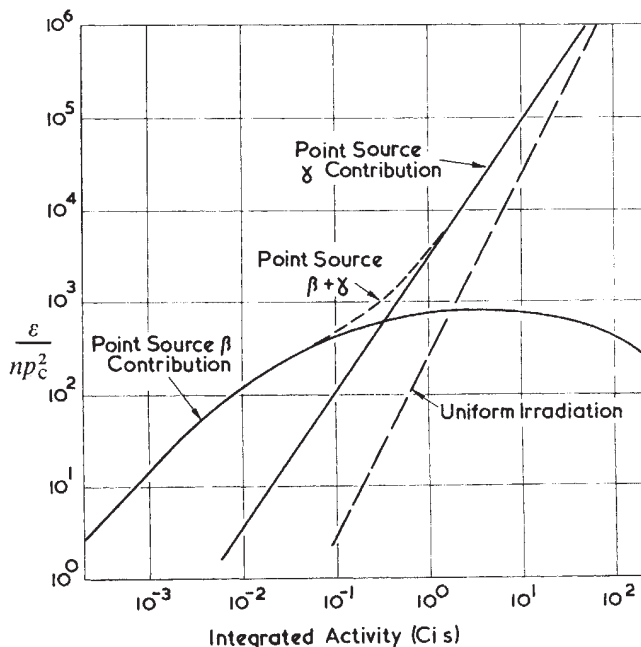


Fig. 2 Expectation in 1 kg of tissue from a point source of ^{60}Co compared with a uniform distribution of the same amount of energy for a cellular response $\phi(p_c D) = p_c^2 D^2 \exp(-0.5 \times 10^{-3} D)$.

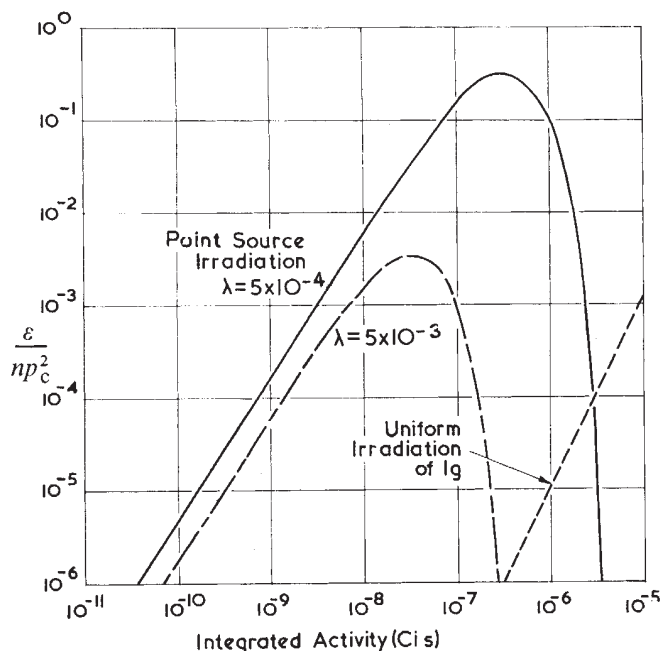
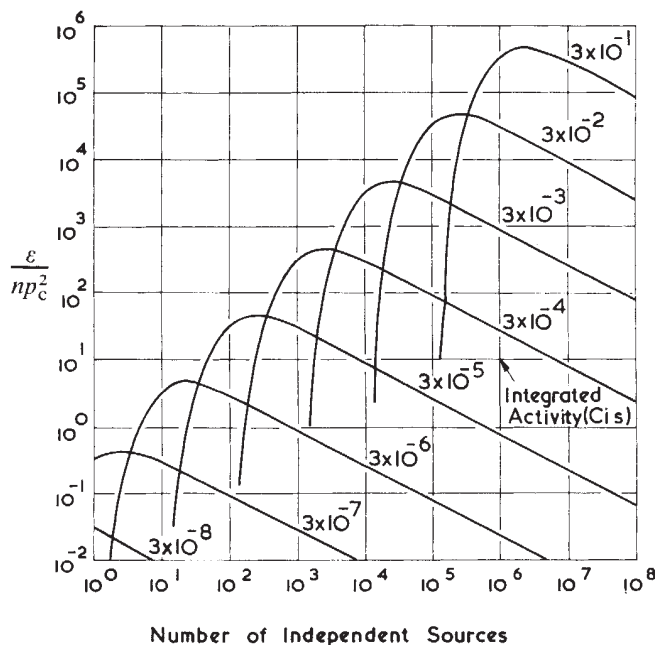


Fig. 3 Expectation from a point source of ^{239}Pu α particles as a function of source activity compared with that from uniform irradiation of 1 g of tissue by the same amount of energy using a cellular dose-response function $\phi(p_c D) = p_c^2 D^2 \exp(-\lambda D)$ for $\lambda = 5 \times 10^{-4}$ and $5 \times 10^{-3} \text{ rad}^{-1}$.

Fractionation of single point sources

Figure 4 shows the results of breaking up a single ^{239}Pu α -emitting 'hot particle' into as many as 10^8 smaller particles, keeping the mean organ dose constant. We see that there is a maximum expectation when each particle activity approaches that corresponding to the peak of the single point source results of Fig. 3. Further fractionation decreases the expectation, since the differential coefficient of the single point source expectation, with respect to source strength $< 10^{-7} \text{ Ci s}$,

Fig. 4 Expectation in a large mass of tissue for a given organ burden of ^{239}Pu fractionated to varying numbers of independent sources. The cellular response function peaked at 4,000 rad.



is > 1 . The maximum expectation increases roughly linearly with increasing mean organ dose.

Figure 5 shows the results of fractionation of the single β -emitting point source using the response function peaking at 4,000 rad. For the ^{86}Rb point source, at low mean organ doses, the effect of increasing the number of sources while keeping the organ burden constant, is to reduce the risk. Above $\sim 10 \text{ Ci s}$ (4 rad to 1 kg) there is a 'pessimum' number of particles. At higher doses significant dose overlapping occurs between sources. When the ^{35}S single point source is fractionated, the expectation is increased for mean doses of more than 3×10^{-4} rad to 1 kg (10^{-2} Ci s). We note the four orders of magnitude lower mean organ dose at which fractionation becomes important for the low, as compared with the high-energy β emitter.

Moving point sources of ^{239}Pu

As our model is assumed to be independent of dose rate, the problem of a moving point source is essentially that of a line source. We have previously presented a theoretical model for such β sources⁶ and have now performed similar integrations for a ^{239}Pu α -point source moving various distances through tissue. Neglecting end effects, the expectation for a source strength S_L per unit length (that is the integrated source strength/velocity of travel) is shown in Fig. 6. We see that a high source strength moving with high velocity can be equivalent in expectation to a low source strength at low velocity.

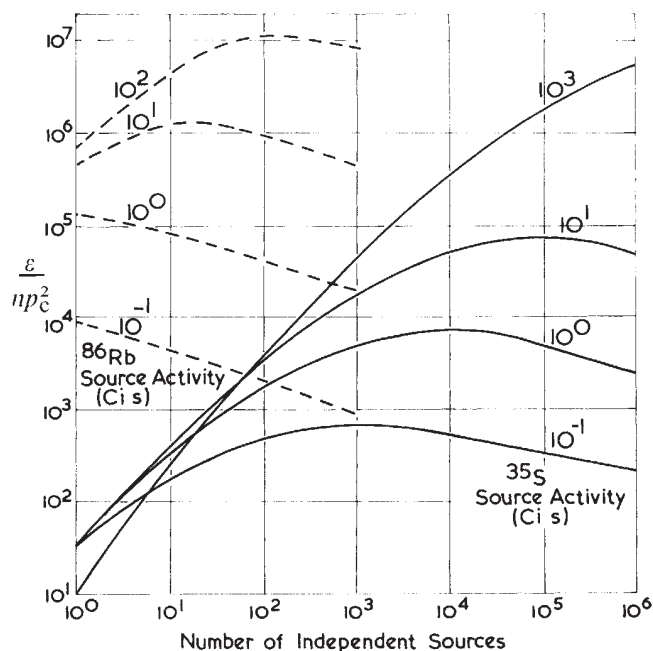


Fig. 5 Expectation in a large mass of tissue as a function of the number of discrete sources for a given organ burden of a high (^{86}Rb) and low (^{35}S) energy β emitter, assuming $\phi(p_c D) = p_c^2 D \exp(-0.5 \times 10^{-3} D)$.

These results again merely reflect the fact that the significant contributions to expectation originate largely from a narrow dose band around the maximum of the dose-response curve.

Absolute values of expectation

The basic problem⁶ lies in attempting to quantify p_c , the probability of transformation per cell per rad. We know that for a range of human body organs the risk of carcinogenesis is between $\sim 0.1\%$ and 1% per 100 rad, for doses in the region of a few hundred rad (ref. 13). If a typical value of 0.5% per 100 rad be assumed, on the basis of a linear hypothesis the pro-

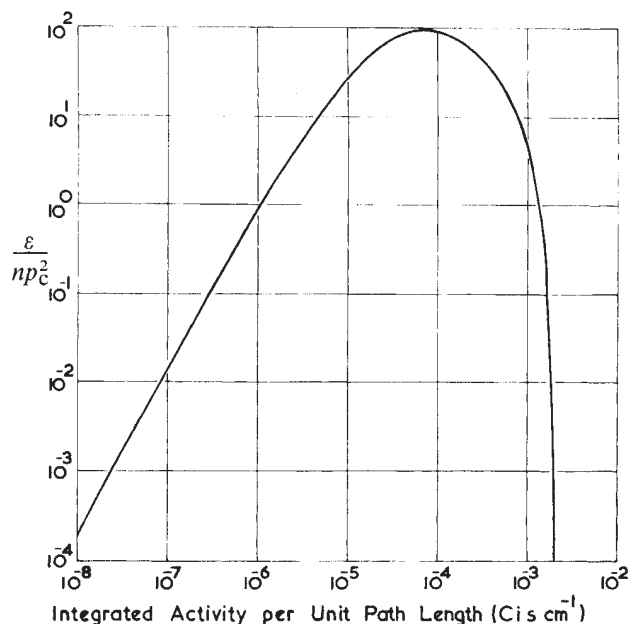


Fig. 6 Expectation from a moving point source of ^{239}Pu using a cellular response function, $\phi(p_c D) = p_c^2 D^2 \exp(-0.5 \times 10^{-3} D)$.

bability per tissue (p_t | linear) is

$$(p_t | \text{linear}) = 5 \times 10^{-5} \text{ rad}^{-1}$$

On a dose-squared hypothesis, using equation (2) and ignoring the exponential term at the normalisation dose of 100 rad

$$(p_t | \text{square}) = 7.1 \times 10^{-4} \text{ rad}^{-1}$$

Assuming an organ of mass M with n cells per unit mass it is easy to show that

$$(p_c | \text{linear}) = (p_t | \text{linear}) / Mn$$

$$(p_c | \text{square}) = (p_t | \text{square}) / (Mn)^{1/2}$$

For a 1-kg organ containing $\sim 10^9$ cells per unit mass, we find

$$n(p_c | \text{square})^2 = 5 \times 10^{-10} \text{ g}^{-1} \text{ rad}^{-1}$$

On the linear hypothesis the value is

$$n(p_c | \text{linear}) = 5 \times 10^{-8} \text{ g}^{-1} \text{ rad}^{-1}$$

We note that normalisation at a higher dose would reduce the estimate of $n(p_c | \text{square})^2$.

Respirable 'hot particles' often have activities in the range 10 pCi–100 nCi. Let us assume a mean residence time in the lung of 10^8 s (> 3 yr). The results then obtained for a single point source of ^{86}Rb and ^{35}S are shown in Table 1, where it is seen that the absolute expectation for a high energy β emitter (^{86}Rb) from a point source using the square-law response function is essentially the same as that predicted by the linear hypothesis using mean organ dose. For a low energy β emitter (here ^{35}S), at higher source strengths, assumed linearity leads to higher values of the absolute predicted risks.

If the results were evaluated for 1 g of tissue, we should raise both $n(p_c | \text{linear})$ and $n(p_c | \text{square})^2$ by a factor of 10^9 . In Table 1 the mean organ doses and absolute expectations would also increase by a factor of 10^9 .

When fractionation is considered, at organ burdens < 10 Ci s the ^{86}Rb effects are reduced, so the use of mean organ doses and linearity overestimates risks. For ^{35}S , however, fractionation increases expectation: at 10 Ci s an increase of 10^3 in expectation is seen on the square-law response. The effect of this on the ^{35}S expectations in Table 1 is to bring the linear hypothesis and power-law estimates into close agreement.

The results for absolute expectations associated with stationary sources of ^{239}Pu α particles are shown in Table 2. Since the single point-source results apply only at very low mean organ organ dose ($< 10^{-6}$ Ci s $= 3 \times 10^{-6}$ rad kg $^{-1}$), expectations have been quoted for the distribution of activity leading to peak expectation following fractionation (Fig. 4). We see that

Table 2 Comparison of absolute expectation from ^{239}Pu α -point sources fractionated to give peak expectation with the cellular response function $\phi(p_c D) = p_c^2 D^2 \exp(-0.5 \times 10^{-3} D)$, and that predicted by linearity for a 1-kg organ and a given incidence of 0.5% at 100 rad

Integrated activity (Ci s)	D to 1 kg (rad)	Absolute expectation	
		Peak value, nonlinear response	Linear response
10^{-7}	3×10^{-7}	7.5×10^{-11}	1.5×10^{-11}
10^{-3}	3×10^{-3}	7.5×10^{-7}	1.5×10^{-7}
10^{-2}	3×10^{-2}	7.5×10^{-6}	1.5×10^{-6}
10^{-1}	3×10^{-1}	7.5×10^{-5}	1.5×10^{-5}
10^0	3×10^0	7.5×10^{-4}	1.5×10^{-4}
10^1	3×10^1	7.5×10^{-3}	1.5×10^{-3}

for virtually all source strengths of interest in radiological protection, the peak of absolute expectation predicted by the power-law response is a factor of ~ 5 above the value estimated by using mean organ doses and linearity. To obtain such expectations it has been necessary to choose circumstances when the ^{239}Pu α energy is distributed in such a way that the largest number of cells are exposed to doses in the region of the peak of the dose-response curve.

The moving α -particle source results from Fig. 6 have been transformed into absolute expectations in Table 3. Once again the use of linearity and mean organ doses predicts expectations

Table 1 Comparison of absolute expectation from a single point source of ^{35}S or ^{86}Rb in a 1-kg organ, as predicted using cellular responses $\phi(p_c D) = p_c^2 D^2 \exp(-0.5 \times 10^{-3} D)$ and $p_c D$ (linearity) using different values of p_c derived for each response function assuming a given incidence of 0.5% per 100 rad

Nuclide	Integrated activity (Ci s)	ϵ / np_c^2 (Fig. 1)	D to 1 kg (rad)	Absolute expectation	
				Nonlinear response	Linear response
^{86}Rb $E_\beta = 0.67$ MeV	10^{-3}	2×10^1	41.4×10^{-4}	1.0×10^{-8}	2×10^{-8}
	10^{-2}	4×10^2	4.14×10^{-3}	2.0×10^{-7}	2×10^{-7}
	10^{-1}	8×10^3	4.14×10^{-2}	4.0×10^{-6}	2×10^{-6}
	10^0	1.2×10^5	4.14×10^{-1}	6.0×10^{-5}	2×10^{-5}
	10^1	4.4×10^6	4.14×10^0	2.2×10^{-4}	2×10^{-4}
^{35}S $E_\beta = 0.05$ MeV	10^{-3}	5×10^0	3×10^{-5}	2.5×10^{-9}	1.5×10^{-9}
	10^{-2}	1.8×10^1	3×10^{-4}	9.0×10^{-9}	1.5×10^{-8}
	10^{-1}	3.3×10^1	3×10^{-3}	1.7×10^{-8}	1.5×10^{-7}
	10^0	3.8×10^1	3×10^{-2}	1.9×10^{-8}	1.5×10^{-6}
	10^1	3.8×10^1	3×10^{-1}	1.9×10^{-8}	1.5×10^{-5}

Table 3 Comparison of absolute expectation from a moving point source of ^{239}Pu α activity using a cellular response $\phi(p_e D) = p_e^2 D^2 \exp(-0.5 \times 10^{-3} D)$ and that predicted by linearity for a 1-kg organ and a given incidence of 0.5% per 100 rad

Source strength S (Ci)	Velocity (cm s^{-1})	S_L (Ci s cm^{-1})	Distance moved (cm)	Absolute expectation Nonlinear response (Fig. 6)	Linear response
10^{-12}	10^{-1}	10^{-11}	10^{-2}	5×10^{-21}	1.5×10^{-17}
			10^1	5×10^{-18}	1.5×10^{-14}
	10^{-5}	10^{-7}	10^{-2}	7×10^{-14}	1.5×10^{-13}
			10^1	7×10^{-11}	1.5×10^{-10}
10^{-9}	10^{-1}	10^{-8}	10^{-2}	10^{-15}	1.5×10^{-14}
			10^1	10^{-12}	1.5×10^{-11}
	10^{-5}	10^{-4}	10^{-2}	5×10^{-10}	1.5×10^{-10}
			10^1	5×10^{-7}	1.5×10^{-7}
	10^{-6}	10^{-3}	10^{-2}	2.5×10^{-11}	1.5×10^{-9}
			10^{-1}	2.5×10^{-8}	1.5×10^{-6}

in excess of those for the power-law response except when the source strength per unit length (or source strength/velocity) is at the peak of the curve in Fig. 6 (at $\sim 10^{-4}$ Ci s cm^{-1}), when the power law predicts slightly higher expectations than the linear hypothesis. This is true for any combination of source strengths, velocities and distances, which again reflects the fact that peak expectations arise from the power-law response when the greatest number of cells are exposed to doses near the peak of the dose-response curve.

Conclusions

We have estimated carcinogenic expectations by integrating nonlinear peaked cellular dose-response functions over spatially varying dose distributions and contrasted them with the expectations predicted by the linear hypothesis. If the dose-response function is peaked, then it is noticeable that the relative expectation is largely controlled by cells receiving doses in the vicinity of the peak. Thus, whether the relative maximum expectation arises from uniform irradiation, from a single point source, from a 'pessimism' number of 'hot particles', or during relative motion of the source, depends on the energy absorbed being distributed so as to subject the largest number of cells to doses near the peak of the response curve.

When we estimate absolute values of expectation, however, the use of the mean organ dose and the assumption of a linear non-threshold dose-response function seems to give a good guide to the upper limit of absolute expectation predicted

even for a peaked nonlinear function. Linearity can be very conservative for small numbers of α - or low energy β -emitting sources, while at worst it seems unlikely to underestimate risks by more than a factor of ~ 5 for those exceptional distributions of activity which lead to maximum expectations. The circumstances in which the 'hot particles' are relatively more dangerous are those in which the absolute risks are low.

An underestimation of the risk on assuming linearity would be most likely to occur if there was a high power-law response (say, $D^4 \exp(-\lambda D)$) fitting the linear response at low doses (< 100 rad) and the response peaking in the region of 10,000 rad rather than between 100 and 1,000 rad.

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- ¹ Recommendations of the ICRP, Publication 9, paras 28, 29 (Pergamon, Oxford, 1966).
- ² Tamplin, A. R., Cochran, T. B., Radiation Standards for Hot Particles (Natural Resources Defence Council, Washington, DC, 1974).
- ³ Lovins, A. B., and Patterson, W. C., *Nature*, **254**, 278 (1975).
- ⁴ The Toxicity of Plutonium, (MRC, HMSO, 1975).
- ⁵ Bair, W. J., Richmond, C. R., and Wachholz, B. W., *A Radiological Assessment of the Spatial Distribution of Radiation Dose from Inhaled Plutonium* (WASH 1320, 1974).
- ⁶ Mayneord, W. V., and Clarke, R. H., *Brit. J. Rad.*, Supplement 12 (1975).
- ⁷ UNSCEAR Ionising Radiation: Levels and Effects, II (UN, New York, E72.1X.18, 1972).
- ⁸ Mole, R. H., *Br. med. Bull.*, **29**, 1, 78-83 (1973).
- ⁹ Cross, W. G., *Tables of β -dose Distributions* (AECL-2793, 1967).
- ¹⁰ Rossi, H. H., and Kellerer, A. M., *Science*, **175**, 200-202 (1971).
- ¹¹ Bair, W. J., Kellerer, A. M., Stannard, J. N., and Thompson, R. C., *Alpha Emitting Particles in the Lung*, NCRP, 531-534 (1976).
- ¹² Spiers, F. W., and Vaughan, J. M., *Nature*, **259**, 531-534 (1976).
- ¹³ Pochin, E. E., *Encyclopaedia of Medical Radiology*, II, 341-355 (1973).

Aspects of the dosimetry of plutonium in bone

M. C. Thorne

Medical Research Council, Radiobiology Unit, Harwell, Didcot, Oxfordshire OX11 0RD, UK.

After animals have been exposed to plutonium it eventually becomes distributed unevenly throughout the bone tissue. There is therefore a need to study the dosimetry of plutonium, particularly ^{239}Pu , as a function of its depth of burial in bone. This article discusses a simple approach to the problem, and the magnitude of some of the possible errors involved.

PLUTONIUM is deposited primarily on the endosteal surfaces of cortical and trabecular bone¹ although a certain amount is also found on periosteal surfaces. After deposition the plutonium gradually moves into the volume of bone mainly by

becoming buried under newly deposited bone mineral¹. Thus, at times long after a single intravenous injection, the distribution of plutonium in bone will typically consist of a thin layer of the element some distance below the endosteal surface together with a diffuse distribution between that layer and the endosteal surface.

Endosteal surfaces are defined here as the mineral bone surface of all marrow cavities and haversian canals; that is, the interface between mineralised bone and non-mineralised osteoid. Thus, if the skeleton contains $I \mu\text{Ci}$ of ^{239}Pu and if $A \text{ m}^2$ is the total area of endosteal surface, then $I/A \mu\text{Ci}$ of the radionuclide will be associated with each m^2 of the surface, if deposition on periosteal surfaces is neglected. If the plutonium is buried a distance, $d \mu\text{m}$, below the endosteal

Table 1 Calculated values $10^3(k(q, d)/q)$

Depth, d (μm)	5	10	15	20	25	30
0	3.10	2.53	2.20	1.94	1.74	1.57
	± 0.22	± 0.16	± 0.12	± 0.09	± 0.07	± 0.06
1	2.78	2.36	2.04	1.80	1.60	1.42
	± 0.11	± 0.09	± 0.07	± 0.06	± 0.06	± 0.05
2	2.24	1.98	1.75	1.56	1.38	1.22
	± 0.11	± 0.08	± 0.06	± 0.05	± 0.04	± 0.03
3	2.14	1.89	1.65	1.45	1.28	1.14
	± 0.11	± 0.09	± 0.07	± 0.05	± 0.05	± 0.04
4	1.97	1.82	1.61	1.44	1.27	1.11
	± 0.10	± 0.09	± 0.07	± 0.05	± 0.05	± 0.04
5	1.84	1.68	1.50	1.31	1.12	0.96
	± 0.05	± 0.04	± 0.04	± 0.04	± 0.04	± 0.04
6	1.64	1.43	1.30	1.14	0.98	0.82
	± 0.06	± 0.04	± 0.04	± 0.04	± 0.04	± 0.03
7	1.70	1.46	1.25	1.08	0.91	0.76
	± 0.10	± 0.08	± 0.06	± 0.05	± 0.04	± 0.04
10	1.22	1.07	0.93	0.79	0.64	0.53
	± 0.05	± 0.06	± 0.05	± 0.04	± 0.03	± 0.03
15	0.74	0.58	0.44	0.33	0.26	0.22
	± 0.04	± 0.04	± 0.03	± 0.02	± 0.02	± 0.02
20	0.31	0.17	0.12	0.09	0.07	0.06
	± 0.04	± 0.02	± 0.02	± 0.01	± 0.01	± 0.01

Errors are 1 s.d. statistical errors arising from the limited number of Monte Carlo iterations used.

surface then the dose rate, $D_s(q, d)$ to an endosteal tissue layer of thickness q μm will be given by

$$D_s(q, d) = I \times E_\alpha \times 51.2 \times k(q, d) / Aq \text{ rad d}^{-1} \quad (1)$$

where E_α is the energy of the ^{239}Pu α emission in MeV, ρ is the density of soft tissue, which may be taken as 1 g cm^{-3} , and $k(q, d)$ is the average fraction of that energy absorbed in an endosteal tissue layer of q μm thickness if the decay occurs d μm below the endosteal surface.

It is usual to define D_{BONE} as the average dose rate to an infinite extent of mineral bone contaminated at a level of $I/M \text{ } \mu\text{Ci g}^{-1}$, M being the mass of mineral bone in grams. Thus

$$D_{\text{BONE}} = 51.2 \times I \times E_\alpha / M \text{ rad d}^{-1} \quad (2)$$

From equations (1) and (2) it can be seen that

$$D_s(q, d) / D_{\text{BONE}} = Mk(q, d) / Aq \quad (3)$$

For man, M may be taken as 5,000 g, since the evidence suggests that bone mineral comprises about 7% of the total adult body weight². The value of A has been variously estimated at 14.0 (ref. 2), 12.5 (J. M. Marshall *et al.*, unpublished) and 16 m² (F. W. Spiers, personal communication). I shall take A at 12.5 m² in accordance with the results presented by Marshall (unpublished), but it should be emphasised that this is an estimate, and that the true mean for a particular occupationally exposed population could easily differ from this by as much as 25%.

Inserting these values into equation (3) gives

$$D_s(q, d) / D_{\text{BONE}} = 400k(q, d) / q \quad (4)$$

The value of $k(q, d)$ has been calculated using Monte Carlo techniques under two simplifying assumptions: first, that bone

surfaces are essentially flat over distances comparable with the range of an α particle in tissue; second, that the surfaces are sufficiently far apart for cross-fire to be neglected. Decays were originated at a distance d μm below the endosteal surface, and emission directions were chosen at random to yield an isotropic decay distribution. Using a modified form of the Bragg equation the rate of energy loss along each of the randomly chosen tracks was evaluated and the fraction of energy deposited in the first q μm above the endosteal surface was thus determined.

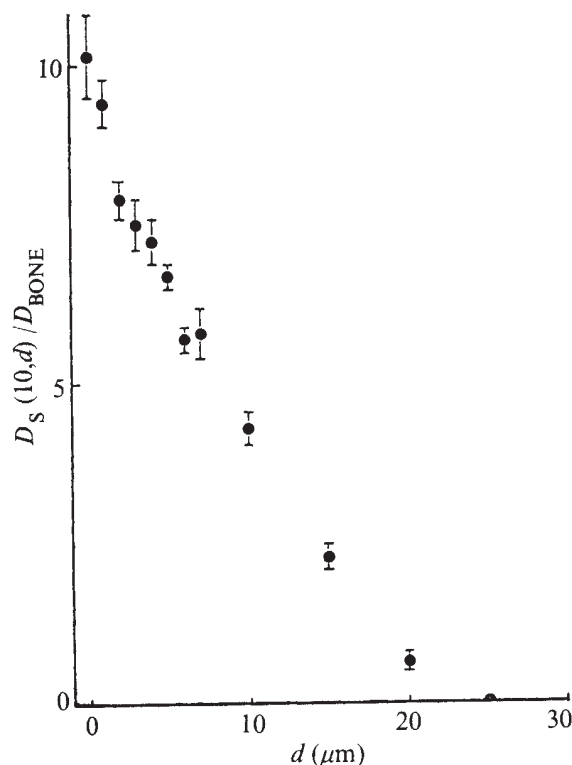


Fig. 1 Relationship between the dose rate to the endosteal layer and the depth of burial of plutonium in bone mineral.

A considerable amount of approximation was involved in determining the rate of energy loss along each track. For soft tissue the form of the Bragg equation was taken from Harley and Pasternak³ except that an α -particle shielding correction was used at energies less than 2 MeV, in order that the calculated values of stopping power in this energy range were in agreement with those tabulated by Walsh⁴. That gave a ^{239}Pu α -particle range in soft tissue of 37 μm . The same Bragg equation was used for mineralised bone, except that, at all α -particle energies, the stopping power was multiplied by a constant, C , given by

$$C = (\rho_{\text{BONE}} / \rho_{\text{SOFT TISSUE}}) (SP_{\text{BONE}} / SP_{\text{SOFT TISSUE}}) \quad (5)$$

The value for ρ_{BONE} , the density of mineral bone, was taken to be 1.95 g cm^{-3} (ref. 2) and that of $\rho_{\text{SOFT TISSUE}}$ was taken as unity. The ratio of the mass stopping power of soft tissue, $SP_{\text{SOFT TISSUE}}$ to the mass stopping power of bone, SP_{BONE} , is not well known. It has been estimated at 1.225

Table 2 Values of $D_A(d) / D_{\text{BONE}}^*$

$d(\mu\text{m})$	0	1	2	3	4	5	6	7	10	15	20	25
	0.83	0.73	0.62	0.57	0.56	0.48	0.41	0.38	0.27	0.11	0.029	0.00
	—	± 0.03	± 0.02	± 0.02	± 0.02	± 0.02	± 0.02	± 0.02	± 0.02	± 0.01	± 0.003	—

*Errors are statistical errors from the number of Monte-Carlo iterations used.

(ref. 5) and 1.41 (J. M. Marshall, unpublished), depending on the degree of calcification assumed. The ratio is taken here as 1.3, but that value could easily be in error by as much as 10%. It is for this reason that the same Bragg curve has been used for both mineral bone and tissue, since any difference depending upon the different elemental composition of mineral bone and soft tissue would be small compared with the estimated systematic error of 10%.

Using the Monte Carlo calculations $k(q, d)/q$ was calculated for various values of q and d (Table 1).

If q is taken as 10 μm , a value suggested⁶ for evaluating the dose to cells on endosteal surfaces, it can be seen from Fig. 1 that the relationship between the dose rate to the endosteal layer and the depth of burial of the plutonium is slightly curvilinear. Over a range of depths of burial from 2 μm to 20 μm this curvilinear relationship may, however, be closely approximated by a straight line. Thus, for plutonium distributions in which the bulk of the plutonium lies within this range, the average depth of burial may be used to calculate the average endosteal dose.

The methods described here have also been used to calculate the average dose rate to active red bone marrow from plutonium buried at different depths in mineral bone. In the adult human, active red bone marrow is primarily associated with the trabecular marrow spaces, and is estimated to have a mass of 1,500 g (ref. 2). If $I_T \mu\text{Ci}$ of ^{239}Pu are associated with trabecular bone and $I_C \mu\text{Ci}$ are associated with cortical bone, then the average dose rate to active red bone marrow, $D_A(d)$, from ^{239}Pu buried a distance $d \mu\text{m}$ below the endosteal surface is given by

$$D_A(d) = 51.2 I_T E_a k(q_A, d) / 1,500 \text{ rad d}^{-1} \quad (6)$$

where q_A is a value of q greater than the range of ^{239}Pu α particles in soft tissue.

Since

$$D_{\text{BONE}} = 51.2(I_C + I_T)E_a/5,000 \text{ rad d}^{-1} \quad (7)$$

It follows that

$$D_A(d)/D_{\text{BONE}} = (10/3)(I_T/(I_C + I_T))k(q_A, d) \quad (8)$$

If ^{239}Pu is uniformly distributed over all endosteal surfaces, then I_C will be approximately equal to I_T , since the endosteal areas of cortical and trabecular bone are approximately equal². Under this assumption

$$D_A(d)/D_{\text{BONE}} = (5/3)k(q_A, d) \quad (9)$$

Values of $D_A(d)/D_{\text{BONE}}$ calculated according to this formula are given in Table 2 for various depths of burial. It should be noted that if the plutonium forms a thin layer exactly on endosteal surfaces then, by simple geometric arguments, $k(q_A, 0) = 0.5$ and $D_A(0)/D_{\text{BONE}} = 0.83$.

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- 1 Vaughan, J., in *Handbook of Experimental Pharmacology*, 36: Uranium, Plutonium, Transplutonic Elements, 400–413 (Springer, Berlin, 1973).
- 2 International Commission on Radiological Protection Publication 23, (1975).
- 3 Harley, N. H., and Pasternak, B. S., *Health Phys.*, 23, 771 (1972).
- 4 Walsh, P. J., *Health Phys.*, 19, 312 (1970).
- 5 Splers, F. W., and Whitwell, J. R., in *Strontium Metabolism, Proc. second Int. Conf. Strontium Metabolism, Glasgow* (National Technical Information Service, Springfield, Virginia, 1972).
- 6 International Commission on Radiological Protection Publication 11, (1967).

Neutralised colliding beam torus

R. M. Kulsrud & D. L. Jassby

Plasma Physics Laboratory, Princeton University, Princeton, New Jersey 08540

We describe a new type of deuterium-tritium fusion reactor, consisting of two dense oppositely-directed D and T plasma components confined in a torus. These components can be formed by neutral-beam injection at moderate energies. The reactor could have significant power gain with relatively lenient plasma confinement requirements.

EXPERIMENTS over the past few years have demonstrated that a deuterium plasma at temperatures of ~ 1 keV can be stably produced and confined in a torus. These experiments, which are directed toward producing a self-sustained controlled thermonuclear reactor, involve temperatures within one order of magnitude of practical reactor values. It is now reasonable to take seriously other proposals for toroidal devices, not necessarily self-sustained, which could conceivably be constructed sooner and which could produce net power.

Two-component torus

One such possibility, which has been generally accepted as feasible, is the two-component torus (TCT)¹⁻⁴. One component of the TCT is a cold tritium plasma (T), which serves as the target for the other component, an energetic confined beam of deuterons (D). The optimal deuteron injection energy is 150–200

keV (somewhat above the peak of the nuclear cross section) to increase the total probability of fusion, integrated over the deceleration of the deuterons by Coulomb interactions with the tritons and the neutralising plasma electrons. If the electron temperature is $\gtrsim 5$ keV, it can easily be shown that the fusion power output exceeds the beam power injected into the plasma¹⁻⁴ (although the electrical power output is still less than the beam power). The electron temperature is maintained against transport and radiation losses by energy transfer from the energetic deuterons. The critical confinement of the electron energy and of the fast deuterons in such a device can be an order of magnitude poorer than confinement times required for a conventional self-sustaining reactor¹⁻⁴, and plans are well advanced to build a prototype TCT.

Colliding beam torus

Here we discuss another type of beam-driven toroidal reactor. We envisage a toroidal plasma in which the D and T ion components both have substantial energies, ~ 40 keV, but move in opposite directions. The ion distributions are maintained by oppositely directed neutral beams of D and T which are injected into the torus and trapped by ion impact, charge exchange, and electron ionisation in the already-existing plasma of energetic D and T components (in steady state). Because of the opposite velocities of the D and T, the relative collision energy is nearly 4 times the energy of each beam, so that it is above the peak of

the D-T cross section (~ 120 keV). This is analogous to using colliding beams in high energy particle accelerators, but with plasmas the densities can be made much larger by continual trapping of the beams in the torus, and collisions occur throughout the entire volume of the torus. We estimate that densities $\sim 5 \times 10^{13} \text{ cm}^{-3}$ may be achieved for realistic toroidal parameters with a few neutral-beam sources of already available types.

We denote the concept of two energetic colliding components as the 'colliding beam torus' or CBT. Its primary advantage over the TCT is the lower beam voltage required. Highly efficient generation of 40-keV neutral beams is already possible, while efficient generation of 150-keV beams is still in an embryonic state. Another advantage of the CBT is that the number of electrons per energetic ion is lower, so that the same electron temperature may be achieved with even poorer confinement of the electron energy than required by the TCT. The lower temperature and injection energy result in smaller plasma size.

Model of the CBT

Let us consider how the CBT plasma may be set up. To trap the injected neutral beams, one must initially have a conventionally formed target plasma in the torus. Injection of the neutral D and T beams into the plasma must gradually replace the cooler plasma with the two energetic components, and finally, as the energetic components themselves slow down on the neutralising background of electrons, the lower energy parts of the beams must be removed much faster than the more energetic parts of the beams. We require that there be very few impurity ions in the torus, since impurities enhance angular scattering of the energetic ions, thereby destroying their directedness. More precisely, we hypothesise that first, the initial target plasma leaves and does not return, second, the plasma remains very pure, and third, the low energy ions leave much faster than the high energy ions. In our calculations on the CBT, we neglect entirely the loss of ions with energies $W > 2T_e$, and assume that the loss rate for $W < 2T_e$ is constant, with a half life τ . Under these hypotheses, we show by detailed calculations that the CBT situation can actually be maintained in steady state by beam injection at moderate energies. Further, we show that the resulting fusion power is quite favourable, relative to the injected-beam power, and that the energy confinement requirements are quite moderate. We indicate that the colliding plasma components can be formed by neutral injection without undue losses. Finally, calculations show that the CBT is stable to velocity-space instabilities.

There is some experimental evidence that our hypotheses can be realised. Experiments in the ATC tokamak show that, by getting the walls, plasma leaving the torus can be captured⁵. The remaining plasma is very pure, and its density will decay nearly to zero, as required for the initial background plasma of the CBT. Similar results have been obtained in Alcator by special discharge cleaning⁶. We hope that similar effects could be produced in larger devices by efficient divertors. Further, beam injection into several tokamaks has been carried out, and the loss time of beam ions is found to be much larger than that of the thermal plasma⁷⁻⁹. Presumably, the losses are produced by micro-instabilities, and small-scale irregularities affect slow ions more than fast ions.

Calculations

Consider an homogeneous plasma of D and T ions and an equal density of electrons in a uniform magnetic field, $\mathbf{B}$. Let the electrons have a Maxwellian distribution with temperature T_e determined by energy balance. Let there be equal monochromatic sources $S/2$ of D and T at $+v_{20}\mathbf{B}/|\mathbf{B}|$ and $-v_{30}\mathbf{B}/|\mathbf{B}|$ respectively, and let both ion species be uniformly lost for energies $< 2T_e$ at a rate τ^{-1} . Then, for any T_e , a uniform steady state will be reached under the influence of D-T, D-e, and T-e

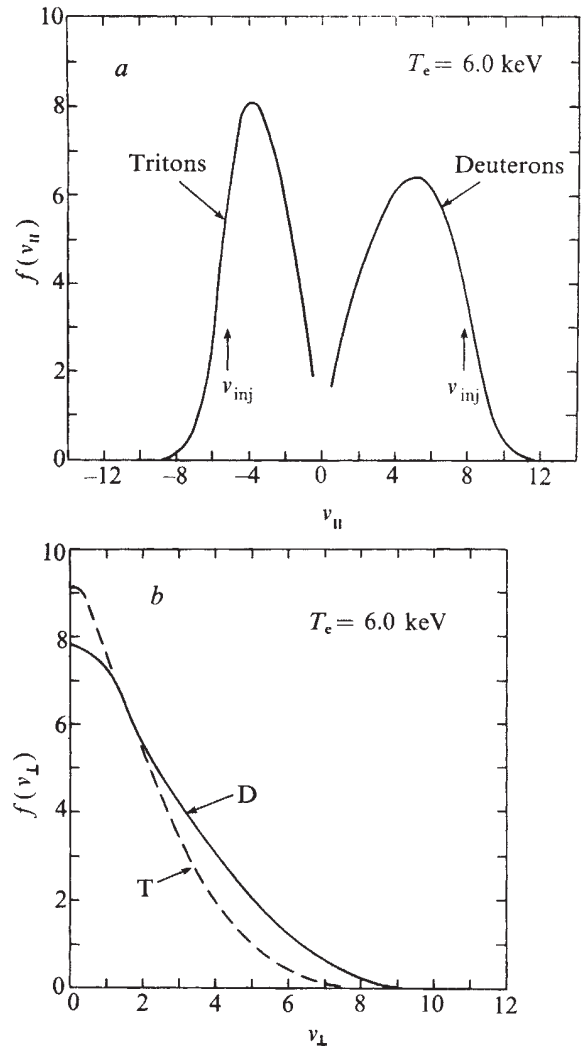


Fig. 1 Steady-state ion velocity distribution functions (a) parallel and (b) in a direction, $\mathbf{x}$, perpendicular to $\mathbf{B}$. The velocity scale is in $(\text{keV})^{1/2}$ for the deuterons only. Injection velocities are indicated by v_{inj} $n_T/n_D = 0.92$.

Coulomb collisions. To determine the resulting three-dimensional distribution functions in velocity space for D and T, $f_2(\mathbf{v}_2)$, $f_3(\mathbf{v}_3)$, we solve two simultaneous Fokker-Planck equations numerically, using one of the standard codes suitably modified to include two species of ions¹⁰. The finite size of the torus is simulated by an energy loss rate τ_E^{-1} produced by thermal conductivity, radiation, and so on. The electron temperature T_e is determined by a power balance between the rate of energy gain by D-e and T-e collisions and the loss rate τ_E^{-1} . In steady-state conditions

$$\frac{dT_e}{dt} = C_2(W_2 - 3/2T_e) + C_3(W_3 - 3/2T_e) - T_e/\tau_E = 0 \quad (1)$$

where W_2 and W_3 are the mean D and T energies, and C_2 and C_3 are constants. We use equation (1) to determine the τ_E required to achieve a given T_e . Since τ , the time constant for cool-ion loss, is somewhat arbitrary, we can make τ_E and τ equal. (The results are, however, relatively insensitive to τ/τ_E in the range 0.5–2.0.)

The resulting distribution functions for $T_e = 6.0$ keV are shown in Figs 1 and 2 for injection energies $W_{20} = 60$ keV for D and $W_{30} = 40$ keV for T. Figure 1a shows the distribution of the parallel component of D and T and Fig. 1b the distribution

in the cartesian component v_x , where x is perpendicular to B . Figure 2 shows the contours of the axisymmetric three-dimensional distribution functions in coordinates $v_{||}$ and $v_{\perp}$, and we see that two oppositely moving axisymmetric ion components are formed. Further, the relative velocities in each beam are small, so that self-collisions dominate and the two components have, approximately, shifted Maxwellian distributions. Although the distribution in pitch angle $\mu \approx \tan^{-1}(v_{\perp}/v_{||})$ is not negligible, angular spread does not disturb the oppositely directed character of the two beams.

Power generated

To find the relative gain in fusion power to beam power, we compute the ratio

$$Q = \frac{E_f}{0.5S(W_{20} + W_{30})} \int f_2(v_2) f_3(v_3) \sigma_{DT} \times \quad (2)$$

$$\times \sigma_{DT}(|v_2 - v_3|) |v_2 - v_3| dv_2 dv_3$$

where σ_{DT} is the D-T nuclear fusion cross section, and $E_f \equiv 17.6$ MeV is the energy released per fusion. The results are given as a function of T_e in Fig. 3a. In Figure 3b we give the

Fig. 2 Steady-state contours of the ion speed distribution function in DT. The deuterons (a) are injected at 60 keV, $\theta = 4^\circ$, and the tritons (b) at 40 keV, $\theta = 176^\circ$. $f(v)$ is normalised to the value at the source. Ions decelerated to energies $< 2T_e$ are assumed to have a lifetime $\tau \approx \tau_E$, as given in Fig. 3b. Velocity units are relative.

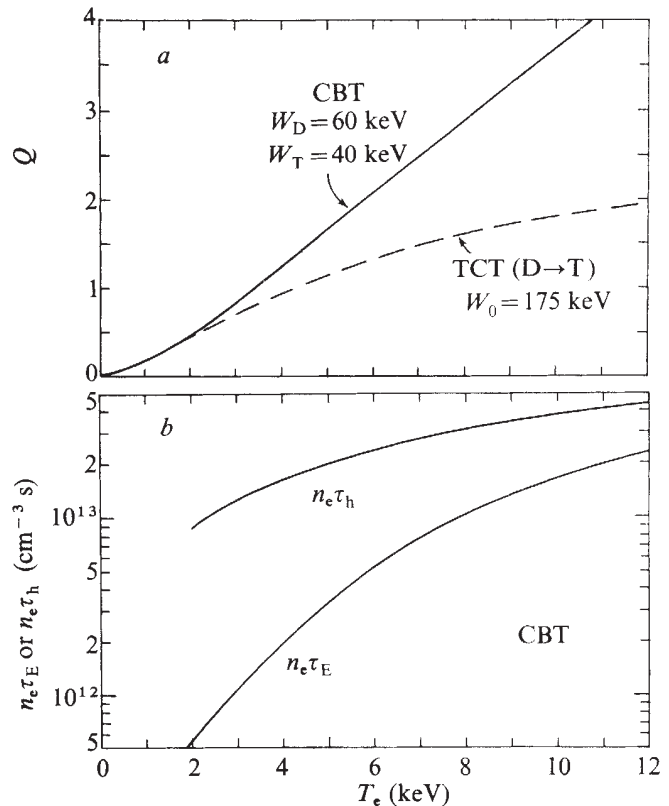
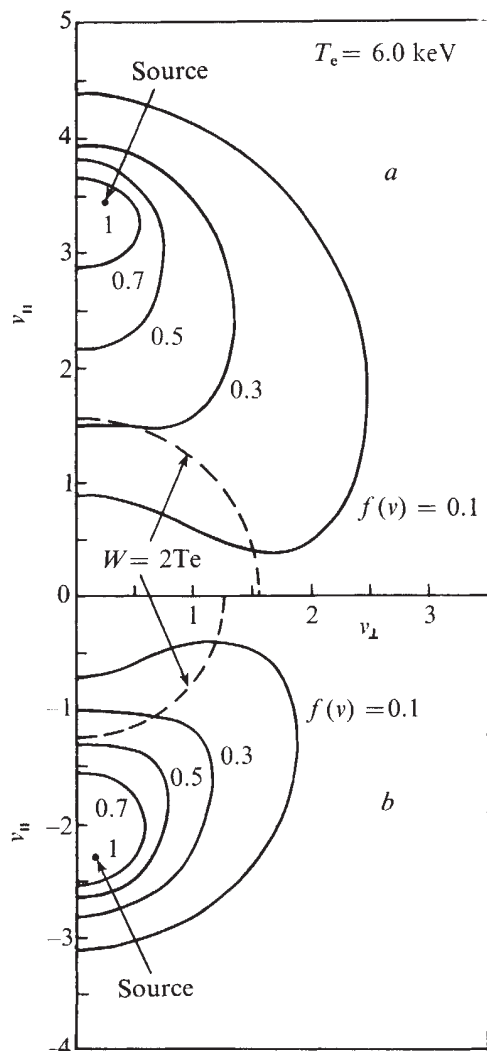


Fig. 3 a, Fusion energy gain Q of the CBT for steady-state operation in DT (17.6 MeV per reaction). b, The confinement parameters ($n_e \tau_E$ and $n_e \tau_h$); τ_h is the total lifetime of injected ions, τ_E is the electron energy confinement time, as well as the lifetime of ions after deceleration below $2T_e$.

confinement parameter $n_e \tau_E = n_e \tau$ required for these T_e . It is seen that Q is larger than unity for electron temperatures in excess of 3.5 keV, and that the required $n_e \tau_E$ for 'breakeven' is 1.5×10^{12} s cm $^{-3}$. The Q values for the TCT are given for comparison. Also shown is $\tau_h = (n_2 + n_3)/S$, the required lifetime of the energetic ions; this value is appreciably larger than τ_E .

In order that the reactor have net electrical power output, Q must satisfy the relationship $Q > (\eta_b \eta_i)^{-1} - 1$, where η_b is the efficiency of neutral-beam production, and η_i is the energy conversion efficiency in the blanket cooling system. Taking $\eta_i = 0.35$, and $\eta_b = 0.7$ (which is quite feasible for low-energy beams¹¹), we require $Q > 3.0$, so that $T_e \geq 8.5$ keV, and $n_e \tau_E \geq 1.3 \times 10^{13}$ s cm $^{-3}$ (Fig. 3). A practical power producer may have to operate at somewhat larger values of $n_e \tau_E$. These conditions can, however, be greatly relaxed if a fissionable blanket is used, and even at $Q \approx 1$, the device could be useful as a fusion test reactor.

In this analysis, we have neglected the effect of 3.5-MeV alpha particles resulting from fusion. Confined alphas will give up their energy almost entirely to the electrons, thereby allowing the same T_e to be obtained with a smaller $n_e \tau_E$. The required $n_e \tau_E$ is reduced from the value in Fig. 3 by the factor $(1 + 0.2\alpha Q)^{-1}$, where α is the fraction of alphas that slow down in the plasma.

Plasma formation and stability

We now consider the formation of the energetic-ion distributions. Neutral beams of D and T are injected into the torus, and ionised. The relative velocity of the incoming D beam is small compared with the D^+ velocity, so that charge exchange on D^+ and ion impact ionisation on T^+ are probably about equal. Charge exchange leads to an energetic secondary neutral of roughly half the energy of the injected neutral. Its loss would

reduce the efficiency of beam trapping by 25%, but estimates show there is a good chance that it too will be trapped by ion impact. Thus, the efficiency of trapping is probably reduced by not more than 10%.

The stability of the CBT plasma has been examined against homogeneous electromagnetic and electrostatic waves. We approximate the equilibrium by two shifted Maxwellian distribution of identical ions. We find stability against all electrostatic instabilities not in the neighbourhood of the ion cyclotron frequency, if the random temperature of each beam exceeds $T_e/2$, a criterion well satisfied by the CBT distribution functions. Thus the CBT is stable against the two-stream instability. The analysis of electrostatic instabilities near the ion cyclotron frequency is more complex, but detailed calculations indicate that the CBT is stable against both electrostatic and electromagnetic waves in this frequency range (F. W. Perkins, unpublished). Finally, a simple analysis approximating the beams by delta functions indicates stability against all electromagnetic modes not near the cyclotron frequency if the plasma energy density is smaller than the energy density of the confining magnetic field—a condition always satisfied. It appears, therefore,

that the steady-state CBT system is stable to all velocity-space modes.

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Received September 16, 1975; accepted January 16, 1976.

- ¹ Dawson, J. M., Furth, H. P., and Tenney, F. H., *Phys. Rev. Lett.*, **26**, 1156–1159 (1971).
- ² Demirkhanov, R. A., Kursanov, Y. B., and Skripal, L. P., *Sov. Atom. Ener.*, **34**, 490 (1973).
- ³ Pistunovich, V. I., *Sov. Atom. Ener.*, **35**, 11 (1973).
- ⁴ Furth, H. P., and Jassby, D. L., *Phys. Rev. Lett.*, **32**, 1176–1179 (1974).
- ⁵ Stott, P. E., Daughney, C. C., and Ellis, R. A., Jr., *Nucl. Fusion*, **15**, 431–440, (1975).
- ⁶ Boxman, G. J., et al., *Proc. seventh Eur. Conf. Controlled Fusion Plasma Physics (Lausanne)*, **11** (1975).
- ⁷ Bol, K., et al., *Plasma Physics and Controlled Nuclear Fusion Research, Proc. fifth Int. Conf., Tokyo 1974, paper CN-33/A4-1*.
- ⁸ Berry, L. A., et al., *ibid.*, paper CN-33/A5-2.
- ⁹ Cordey, J. G., et al., *Nucl. Fusion*, **15**, 444–452 (1975).
- ¹⁰ Killeen, J., and Marx, K. D., in *Methods in Computational Physics*, **9**, 421–489 (Academic, New York, 1970).
- ¹¹ Hovington, J., and Moir, R. W., *Nucl. Fusion*, **14**, 629–637 (1974).

letters to nature

Anti-correlated hard and soft X-ray intensity variations of the black-hole candidates Cyg X-1 and A0620–00

EXTENDED spectral measurements are now available at more than one time epoch for the powerful Monoceros transient, A0620–00, discovered by Elvis *et al.*¹, and the binary source Cyg X-1. We here point out a basic similarity in the unusual spectral time variations of these sources. Both are black-hole candidates, Cyg X-1 by virtue of its orbital dynamics and A0620–00 as suggested by arguments based on the Eddington limit¹ and distance estimates of the optical counterpart. Thus the X-ray emission mechanism could be common.

The type of data obtained by the Ariel V satellite are summarised in Table 1. All the detectors use some form of spin modulation to eliminate background. In particular, measurements with the ST detector are made at two directions offset from the source position given by the RMC. The observed phase of the modulation is then used to confirm that the signal is being correctly detected.

Observations at a few keV indicate that Cyg X-1 switches between two states: a normal state at a low intensity, the

'low state', and an enhanced or 'high state'. Around 1975 May 9 a 'high' to 'low' transition was observed (see ref. 2). Figure 1 shows ST data for the 'high' state taken during May 4–10 and for the 'low' state during May 11–15. The error bars correspond to 1 s.d., determined by counting statistics, which dominates calibration and other systematic errors. Also shown are the CPC data given in ref. 2 for the 'high' and 'low' state. Note that while below ~ 7 keV the 'high' state indeed corresponds to a higher intensity, the change in intensity at higher energies, extending out to at least 150 keV, is in the opposite sense. During the 'low' state a power law

$$\frac{dN}{dE} = 3.3 E^{-1.81} \text{ photons cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$$

(dashed line) represents most of the data, while during the 'high' state, a more complicated spectral form is required.

Qualitatively similar time variations are noticed for A0620–00, both within a restricted low-energy range during the initial turn-on (Ricketts *et al.*³), and now over a much wider energy range, as indicated by the ST observations during the decay phase. Figure 2 shows spectra at two epochs.

Table 1 Ariel V data

Detector and abbreviation	Area (cm ²)	Detector axis tilt to satellite spin axis	Energy range (keV)	Institute and reference
CsI scintillator telescope (ST)	8	3°	26–1200	Imperial College Space Physics Group
Rotation modulation collimator (RMC)	102	0°	1.9–12	MSSL/Birmingham Dr G. Carpenter X-ray Astronomy Group, Birmingham University (personal communication)
Collimated proportional counter (CPC)	100	1.75°	1.5–20	Mullard Space Science Lab. Sanford <i>et al.</i> ²
Sky survey experiment (SSE)	290	$\approx 90^\circ$	2–18	Leicester University X-Ray Astronomy Group Ricketts <i>et al.</i> ³

The first is 1975 mid-August when A0620-00 was near maximum intensity. The SSE data for August 22 and ST data for August 20-23 shown in Fig. 2 can be fitted with a single power law spectrum (solid line)

$$\frac{dN}{dE} = 3.5 \times 10^3 \times E^{-4.7} \text{ photons cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}$$

up to ~ 60 keV, with an indication of hardening > 30 keV to an exponent of ~ -2 . Data for the second epoch in Fig. 2 are from the period October 16-19 for the ST detector, and October 19 for the RMC detector. Error bars for the ST data are again 1 s.d. from counting statistics, and systematic errors are expected to be small compared with this. We now note that whereas the mid-October soft X-ray intensity has decreased by a factor 5 or more, the 20-200 keV intensity seems to have increased by at least a factor 2. Moreover, a spectral break with two power laws is now definitely required to satisfy the totality of the October data. We suggest that the similar

spectral transitions illustrated in Figs 1 and 2 indicate comparable production mechanisms for Cyg X-1 and A0620-00.

In seeking to understand the observed time variations we suggest that the inverse Compton source model of Eardley *et al.*⁴ for Cyg X-1, and by implication A0620-00, could provide a plausible mechanism. Carpenter *et al.*⁵ suppose acceleration in an optically thin region of a source containing a hot electron gas operates like the Fermi mechanism⁶, with a fractional photon energy change per collision

$$\frac{dE}{E} \approx \frac{v^2}{c^2}$$

for electron velocity v . If the probability of escape of photons from the acceleration region, after N collisions, is proportional

Fig. 1 Cyg X-1 X-ray spectra before and after the 1975 May 9 'high' to 'low' state transition measured by Ariel V detectors. The solid curves represent the data of Sanford *et al.*² while the dashed curve represents a power law fit.

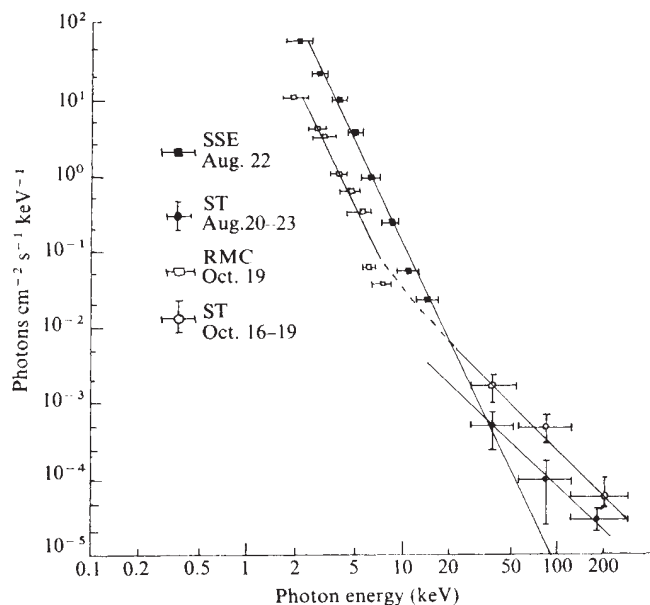
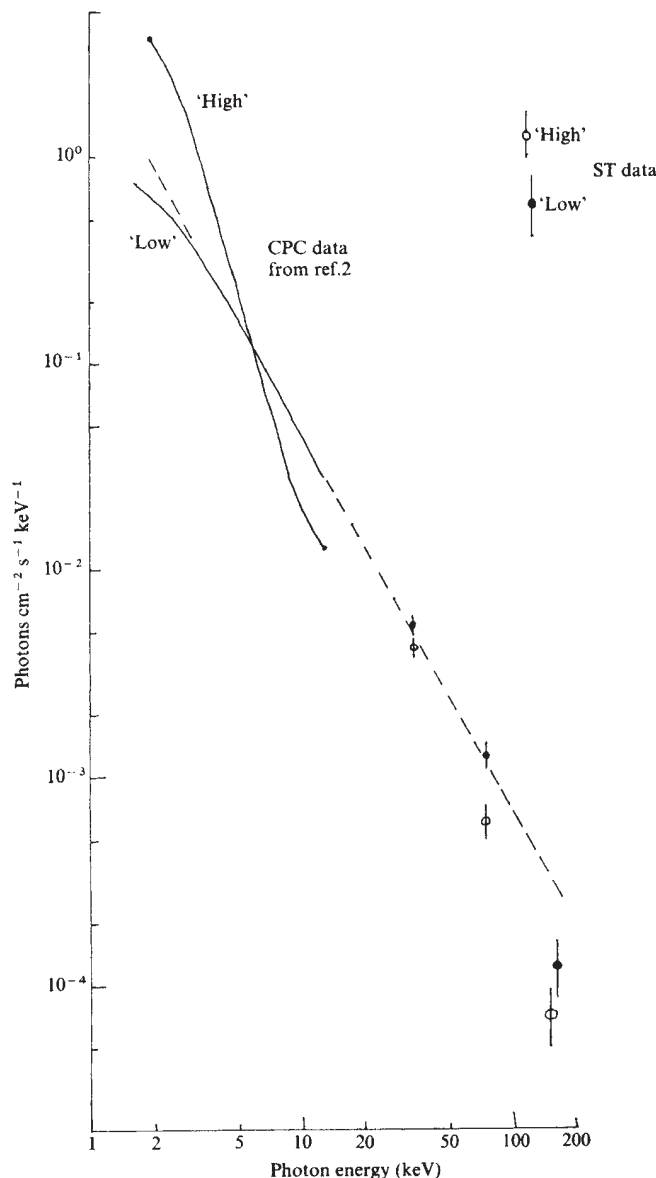


Fig. 2 A0620-00 X-ray spectra in mid-August 1975 and mid-October 1975, observed by Ariel V detectors. Various power law fits are indicated by the solid lines.

to $\exp(-N/k)$, where k is a constant, a power law photon spectrum will result. Changes in the low energy photon supply and in the photon diffusion parameters which could vary in different acceleration regions may account for the observed slope changes and breaks in the spectra of Figs 1 and 2.

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M. J. COE
A. R. ENGEL
J. J. QUENBY

*Cosmic Rays and Space Physics Group,
Blackett Laboratory,
Imperial College,
London SW7 2BZ, UK*

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- ¹ Elvis, M., Page, C. G., Pounds, K. A., Ricketts, M. J., and Turner, M. J. L., *Nature*, **257**, 656 (1975).
- ² Sanford, P. W., Ives, J. C., Bell-Burnell, S. J., Mason, K. O., and Murdin, P., *Nature*, **256**, 109 (1975).
- ³ Ricketts, M. J., Pounds, K. A., and Turner, M. J. L., *Nature*, **257**, 658 (1975).
- ⁴ Eardley, D. M., Lightman, A. P., and Shapiro, S. L., *Astrophys. J. Lett.*, **199**, L153 (1975).
- ⁵ Carpenter, G. F., Coe, M. J., Engel, A. R., and Quenby, J. J., *Proc. R. Soc.* (in the press).
- ⁶ Fermi, E., *Phys. Rev.*, **75**, 1169 (1949).

X-ray transient source at high galactic latitude and suggested extragalactic identification

THE discovery of a number of bright, transient X-ray sources in the Milky Way has been a feature of the first year in orbit of the Ariel V satellite (see, for example, refs 1-4). It is becoming clear from the all-sky data obtained by the Ariel V Sky Survey Experiment (SSE) that at least two distinct classes of (fainter) high latitude transient X-ray source also exist. A particularly interesting first example is reported here.

The SSE detector used in these observations has an effective area at 2-18 keV of 290 cm², and sweeps around the satellite spin plane with a fan-beam collimator of $0.7^\circ \times 10^\circ$ (FWHM), the larger dimension inclined at 65° to the plane. An X-ray source that is detected may be located along a 'line of position', determined by the instantaneous position of the collimator field of view. The width of this 'line of position' is set by the errors in the measurement and in the transfer from spacecraft to celestial coordinates and is a measure of the uncertainty in the source position. A significant improvement in the overall source location can generally be obtained by carrying out a 'cross scan', to obtain an ideally perpendicular 'line of position'. Further details of the SSE and its operation will be published elsewhere.

Our observations are summarised in Fig. 1 and record the discovery of a new X-ray source in Ursa Major, 65° from the galactic plane, in late May, 1975. No source has previously been reported in this region but an extended observation with the SSE during 1975 May 3-9 produced a 3σ signal corresponding to 1.2 Ariel counts s⁻¹ ($\equiv 3.3$ Uhuru counts s⁻¹ for a Crab-like spectrum). It was not visible, at a 2σ upper limit of 1 Ariel count s⁻¹, during 1975 May 9-18 but was present at a substantially higher intensity when the SSE again viewed this region on May 22. Continuous observation to June 1 showed the source to intensify further to a peak ~ 16 counts s⁻¹ on May 28 and then fade in an irregular manner over the following 4 days, while the source remained in the SSE field of view. Further extended observations in mid June, early July and again at the end of August showed the source to be still faintly visible at ~ 0.7 counts s⁻¹. Since the Ariel V Sky Survey is in general deeper than any previous X-ray survey, it cannot yet be ruled out that the source has a normal X-ray intensity of ~ 0.7 counts s⁻¹. If so, it should perhaps be described as highly variable rather than transient, with an order of magnitude increase over a period of 10-28 d, and an additional 'flare' lasting ~ 1 d, during which the intensity increased by a factor of ~ 2 .

Fig. 1 X-ray light curve of A1103+38. Change of scale of the abscissa at MJD42559. Count-rate error bars are $\pm 1\sigma$. Upper limits are 2σ .

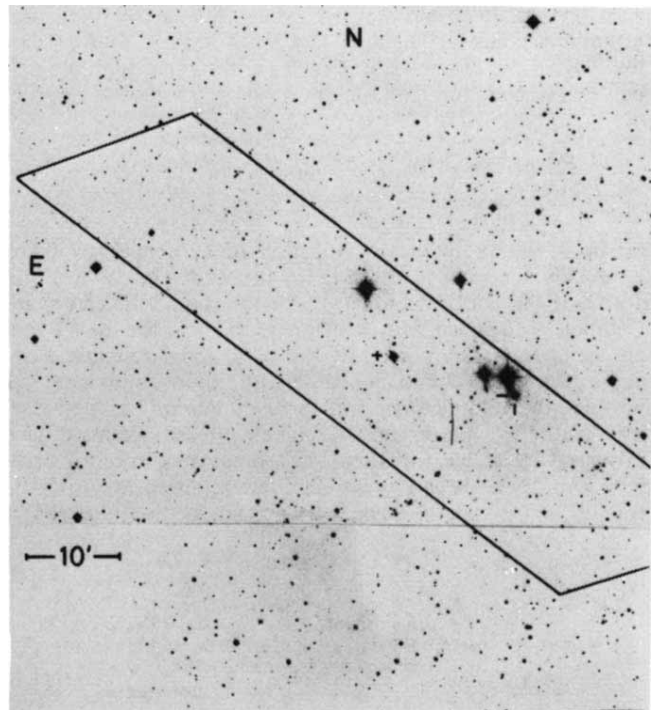
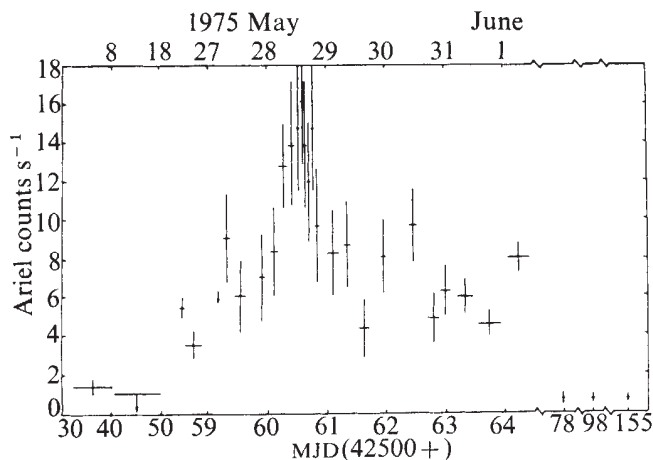


Fig. 2 90% confidence error box of A1103+38 superimposed on a composite of two adjacent, red Palomar Sky Survey prints. The suggested optical candidate MK421 ($\equiv$ B2 1101+38) is indicated lying 0.25° from the centre of the error box (+). The error box has coordinates:

	RA	dec.
Centroid	165.76	38.56
Corners	164.92	38.23
	165.34	38.14
	166.59	38.87
	166.17	38.97

Figure 2 is a copy of the Palomar Sky Survey plate for this region of the sky, on which has been superimposed the 90% confidence error box of the source, designated A1103+38. The narrow dimension of the box represents the line-of-position from the observations of the source near its peak brightness, and includes a statistical error of 0.03° (corresponding to a width of $\pm 2\sigma$, where

$$\sigma = \frac{\text{FWHM}}{\sqrt{6}} \frac{\sqrt{(2N_b + N_s)}}{N_s}$$

and N_b and N_s are the background and signal count rates) plus 0.07° representing the uncertainty in relating the spacecraft attitude to celestial coordinates. The broad (E-W) extension of the error box is from the cross scan line of position where the poorer statistics make the attitude error negligible in comparison.

A preliminary search of the error box for the optical counterpart of A1103+38 has produced three interesting candidates. Two, which are just outside the W corner of the box are IC2615 and IC2619, described in Dreyer's catalogue as faint, round, small and with a brighter middle or nucleus. From this early description it seems possible that either could be distant globular clusters located in the galactic halo (T. A. Matilsky, unpublished). The association of a class of variable, galactic sources with globular clusters has, of course, been firmly established^{5,6}. The third, and preferred, candidate lies within the error box of A1103+38 and only 0.25° from the centroid position. This is the radio source B2 1101+38 (marked in Fig. 2), which has been identified⁷ with the faint elliptical

galaxy MK421, distinguished by an unusually bright and optically variable nucleus. Ulrich has suggested⁸ that this object might be of the BL Lacertae type based on the featureless continuum spectrum. Although later studies⁹ have revealed faint absorption lines from the surrounding galaxy (yielding a redshift of 0.0308), a search of the Harvard archival plates¹⁰ has shown rapid variability of an amplitude greater than for all other BL Lac-type objects, and exceeded only by two QSOs. The proposed identification with A1103+38 is necessarily subjective at this stage and depends mainly on the very unusual nature of MK421 and, in particular, the time scale of its optical variability (>1 mag over several days, ref. 12). The rarity of this type of high latitude X-ray source is also important. To date, it seems unique in its persistence. Several others detected by Ariel V last only hours (B.A.C. *et al.*, unpublished). On the other hand, several of the unidentified high latitude sources in the 3U catalogue are not seen by Ariel V, indicating variability of at least a factor of 5. It seems likely that objects of the BL Lac type are an intermediate class lying between galaxies and QSOs and it is clearly of considerable interest to establish whether, like QSOs, the active nuclei of BL Lac objects are powerful X-ray emitters. With several X-ray satellites now in orbit it should be possible to make more extended observations, coordinated with ground based studies at radio, optical and infrared wavelengths.

We are grateful to Dr M. V. Penston for providing the overlay of Fig. 2 and to the Hale Observatories for permission to reproduce the Palomar chart. The Ariel V project is supported by the Science Research Council who also provide the research post of one of us (M.J.R.).

M. J. RICKETTS
B. A. COOKE
K. A. POUNDS

X-ray Astronomy Group,
Department of Physics,
University of Leicester,
Leicester LE1 7RH, UK

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- ¹ Kaluzienski, L. J., *et al.*, *Astrophys. J. Lett.* **201**, L121 (1975).
- ² Ives, J. C., Sanford, P. W., and Bell-Burnell, J., *Nature*, **254**, 578 (1975).
- ³ Rosenberg, F. D., Eyles, C. J., Skinner, G. K., Willmore, A. P., *Nature*, **256**, 659 (1975).
- ⁴ Elvis, M. S., *et al.*, *Nature* **257**, 656 (1975).
- ⁵ Giacconi, R., *et al.*, *Astrophys. J. Suppl.*, **27**, 37 (1974).
- ⁶ Clark, G. W., Markert, T. H., and Li, F. K., *Astrophys. J. Lett.* **199**, L93 (1975).
- ⁷ Colla, G., *et al.*, *Astr. Astrophys. Suppl.*, **20**, 1 (1975).
- ⁸ Ulrich, M. H., *Astrophys. Lett.*, **14**, 89 (1973).
- ⁹ Ulrich, M. H., *et al.*, *Astrophys. J.*, **198**, 261 (1975).
- ¹⁰ Miller, M. R., *Astrophys. J. Lett.* **201**, L109 (1975).

Mass determination for the X-ray binary system Vela X-1

THE 6.9 mag B0.5 Ib supergiant HD77581 has been identified as the optical counterpart of the X-ray eclipsing binary system 3U0900-40 (Vela X-1)^{1,2}, which eclipses with a period of 8.95 ± 0.02 d. The discovery of regular X-ray pulses in Vela X-1 has also been reported³, with a mean pulse period of 282.9 s, modulated because of the radial velocity variation of the X-ray pulsar in its orbital motion. This makes Vela X-1 the third X-ray binary system in which the orbits of both the optical and the X-ray component can be studied, and the following orbital parameters have been derived⁴, $e_x = 0.15 \pm 0.05$, $\omega_x = 157^\circ \pm 24^\circ$ and $K_x = 268 \pm 12$ km s⁻¹. Analyses of the light curve of HD77581 have shown that the heating effect is too small to be detected⁵. Furthermore, the star is bright and the spectral lines are not very broad. This means that here the first relatively accurate direct mass determination of both the X-ray and the early-type supergiant components of an eclipsing X-ray binary becomes possible.

Earlier studies of the radial velocity variation of HD77581 have given contradictory results^{6,7}. For the semi-amplitude K of the orbit, values between 19 and 40 km s⁻¹ and for the

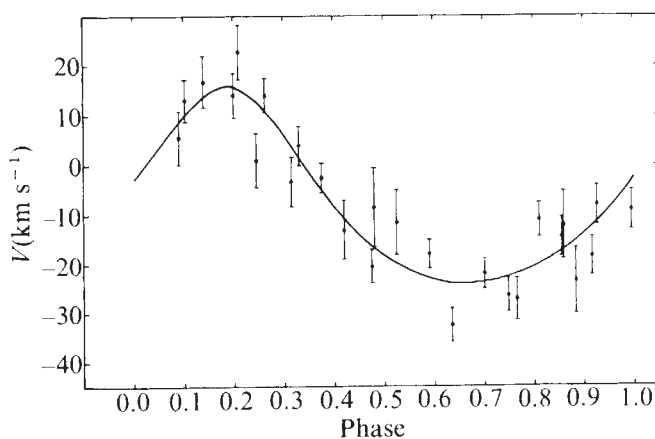


Fig. 1 Radial velocity curve of HD77581 = Vela X-1 for a period of 8.966 d. Phase zero corresponds to mid-eclipse time. The points denote mean values of the measurements of lines of He I and heavier ions. Mean errors per plate are indicated by the length of the vertical bars.

eccentricity from 0.00 to 0.54 have been found. According to Wallerstein⁷, the radial velocity data of HD77581 do not allow a consistent solution of the orbit, because of mass transfer in the system. Here we show that a consistent solution can be obtained, however, provided that the lines of hydrogen—expected to be most sensitive to gas motions in the system—are excluded from the analysis. Our analysis of the radial velocity variations of HD77581 is based on 26 coude spectrograms, obtained with the 152-cm telescope of the European Southern Observatory, La Silla, Chile, in 4 observing runs between April 1973 and June 1975. The spectra were taken on Il-a-0 emulsion, over wavelengths from 3,600–4,950 Å. The dispersion of the plates is 12 Å mm⁻¹ or 20 Å mm⁻¹. The plates obtained in the first observing period⁸ have been remeasured independently for use in this analysis (Fig. 1).

The spectra were measured for line positions with the Grant comparator of the Kapteyn Astronomical Laboratory of the University of Groningen. All absorption features visible in the spectrum were measured, without selecting beforehand a particular set of lines. Some very weak lines were thus missed on some plates, but on the other hand, especially for the weak lines, it was considered an advantage to measure without a

Table 1 Journal of observations

Plate no.	JD2440000+	phase*	v_{rad} (km s ⁻¹)	m.e.	O-C
G3834	1773.626	0.480	-8.64	7.79	+8.00
G3842	1774.608	0.590	-18.02	3.18	+4.77
G3848	1775.582	0.699	-22.03	3.22	+1.76
G3858	1776.583	0.810	-11.00	3.36	+9.25
G3866	1777.613	0.925	-7.72	4.14	+3.93
G3879	1779.535	0.139	+16.74	5.24	+3.11
G3896	1780.632	0.262	+13.85	3.35	+2.68
G3909	1781.631	0.373	-2.37	3.11	+2.18
G3923	1782.556	0.476	-20.48	3.51	-4.18
F1684	2169.522	0.636	-32.65	3.33	-8.88
F1694	2170.522	0.747	-26.57	3.46	-3.76
F1702	2171.477	0.854	-14.37	4.06	+3.25
F1709	2173.593	0.090	+5.68	5.52	-2.80
F1717	2174.581	0.200	+14.16	4.58	-1.39
F1727	2175.594	0.313	-3.27	4.94	-7.56
F1739	2176.559	0.421	-13.00	6.06	-2.36
F1750	2177.492	0.525	-11.60	6.31	+8.22
F1765	2180.463	0.856	-12.23	7.00	+5.23
G6480	2439.647	0.763	-27.27	4.80	-4.98
G6491	2440.710	0.882	-23.62	6.72	-8.12
G6497	2441.734	0.996	-8.92	3.82	-5.24
G6505	2442.684	0.102	+12.98	4.23	+3.03
G6511	2443.658	0.211	+22.75	5.54	+7.51
G6519	2444.729	0.330	+3.80	3.72	+2.08
F3116	2560.509	0.243	+1.18	5.44	-11.90
F3124	2566.531	0.915	-18.34	4.19	-5.73

* Phase zero corresponds to mid-eclipse time JD2441446.54 + $n \times 8.966$ d.

Table 2 Orbital elements for HD77581 = Vela X-1

	Optical observations			
	Mean values for He I and heavier elements	He I	Heavier ions	X-ray pulsar
$P(d)$	8.966	8.966	8.966	8.96
$V_o(km\ s^{-1})$	-7.97 ± 0.82	-8.45 ± 0.64	-7.16 ± 1.06	
$K(km\ s^{-1})$	19.81 ± 1.19	20.54 ± 0.99	21.19 ± 1.42	268 ± 12
e	0.20 ± 0.06	0.23 ± 0.04	0.22 ± 0.07	0.15 ± 0.05
$\omega\ (deg)$	10 ± 17	18 ± 11	2 ± 19	157 ± 24
$a\ sin(i)\ (km)$	$2.4 \pm 0.2 \times 10^6$	$2.5 \pm 0.1 \times 10^6$	$2.6 \pm 0.2 \times 10^6$	$3.27 \pm 0.12 \times 10^7$

predetermined expectation of where lines should be found. Lines of H I, He I, O II, N III, N II, Si III and Si IV were present in at least half of the spectra. From the measurements of the radial velocity of the interstellar Ca II K-line, we found $\bar{v}_{Ca II} = 16.1 \pm 0.6$ (m.e.) and 13.7 ± 1.0 km s⁻¹ for the 12 Å mm⁻¹ and the 20 Å mm⁻¹ spectra, respectively. To increase the homogeneity of the data we reduced the measurements of the 20 Å mm⁻¹ plates to the 12 Å mm⁻¹ system, by applying the correction of 2.4 km s⁻¹.

To get an impression of the internal accuracy of these measurements five plates were measured twice. The differences in the mean velocity obtained from two such measurements of one plate vary between 0.3 and 4.2 km s⁻¹; the s.d.m. per line for one plate varies from 8.0–11.5 km s⁻¹.

In the analysis we used mean values of the radial velocity as obtained from the He I lines and from the lines of heavier ions. These average radial velocities are given in Table 1. A full table of all individual line radial velocity measurements will be published elsewhere.

With the computer program 'Orbit', based on a program of Wolfe *et al.*⁸, the best fitting radial-velocity curve through the points was computed. This was done for all lines of He I and the heavier ions together. Also separate solutions for the He I lines and the heavier ion lines were made. The radial velocity measurements were weighted according to $w_i = 1/\sigma_i^2$, where σ_i is the s.d.m. of the measurements. The average orbital period P determined in these solutions is 8.966 ± 0.005 d, which agrees well with Hutchings' result⁶ ($P = 8.966 \pm 0.001$ d), based on radial velocity determinations by several observers, over a time interval of 17 yr. We therefore subsequently used this period as a fixed parameter in our calculations. The orbital parameters derived from all lines together—except the hydrogen lines—are given in Table 2. The errors quoted in Table 2 are 1σ (68% confidence) limits. The separate solutions for He I and the heavier ions are in good agreement with the mean solution (see Table 2), and give good agreement with the X-ray pulsar data, which for convenience are also given in Table 2. In particular the values of the eccentricity and the angle of periastron, which should be 180° apart for the optical and the X-ray solution, agree well (within the quoted accuracy intervals).

In our opinion this agreement lends confidence to the orbital character of the optical radial velocity variations; the non-orbital (gas streams, stellar wind fluctuations) component does not seem to have much effect on the results from He I and heavier elements. Using $a_{opt} \sin i = 2.5 \pm 0.2 \cdot 10^6$ (1 σ) as a compromise value, we find the following values for the system parameters

$$\begin{aligned} \text{mass ratio: } \mathcal{M}_{opt}/\mathcal{M}_X &= (a_X/a_{opt}) = 13.1 \pm 1.65 \\ \text{total mass: } (\mathcal{M}_{opt} + \mathcal{M}_X) \sin^3(i) &= 21.6 \pm 2.2 \mathcal{M}_\odot \end{aligned}$$

$\mathcal{M}_X \sin^3(i) = 1.52 \pm 0.22 \mathcal{M}_\odot$ and $\mathcal{M}_{opt} \sin^3(i) = 20.0 \pm 2.1 \mathcal{M}_\odot$. From a detailed analysis of the optical-light variations, Avni and Bahcall⁹ have found that the inclination i should be $> 74^\circ$ to get a consistent picture of both the observed light curve and the duration of the X-ray eclipse. Taking 74° and 90° as lower and upper limits of the angle of inclination we get

$$\mathcal{M}_X = 1.61 \pm 0.27 \mathcal{M}_\odot \text{ and } \mathcal{M}_{opt} = 21.2 \pm 2.6 \mathcal{M}_\odot.$$

All quoted errors in the mass parameters are 90% confidence limits. This result shows that the compact component is very probably too heavy to be a white dwarf.

If it is a white dwarf, its evolutionary history implies that it should consist mainly of carbon and oxygen¹⁰; the upper mass limit¹¹ for such white dwarfs is $\sim 1.4 \mathcal{M}_\odot$. Its most probable mass of $1.61 \mathcal{M}_\odot$ is just consistent with the presently allowed theoretical masses of neutron stars¹² ($\mathcal{M} \leq 1.6 \mathcal{M}_\odot$).

The mass determination of the supergiant allows a test of the theoretically computed evolution of massive stars, through a comparison with theoretical evolutionary tracks. The luminosity of HD77581 can be inferred in two ways. The spectral type and luminosity class (B0.5 Ib) provide, in principle, the absolute magnitude M_v , bolometric correction BC and effective temperature T_{eff} . Using the luminosity calibration of Blaauw¹³ or Keenan¹⁴ we derive $M_v = -5.9 \pm 0.4$ mag. For the bolometric correction, values between 2.4 and 2.6 mag have been given^{15,16}. This gives $M_{bol} = -8.4 \pm 0.5$ mag. For the orbital parameters given here and by Rappaport and McClintock⁴, and assuming a minimum observed eclipse angle of 34° , Avni and Bahcall⁹ derive for the radius of HD77581 $R = 30 R_\odot$. For the effective temperature, values ranging from 22,000 K (ref. 17)–29,000 K (ref. 18) have been given for early B-type supergiants. Adopting $T_{eff} = 25,000 \pm 4,000$ K we find $M_{bol} = -9.0 \pm 0.75$ mag. From a comparison with evolutionary tracks^{19,20} we then find the following values of the evolutionary mass: $\mathcal{M}/\mathcal{M}_\odot = 22 \pm 7$ and 30 ± 10 , respectively; these values, although not very accurate, are consistent with the presently determined value of $21.2 \mathcal{M}_\odot \pm 2.4 \mathcal{M}_\odot$. Therefore, the early-type supergiant in this X-ray binary system does not seem to be particularly under-massive for its spectral type (as has been suggested²¹ for Cygnus X-1).

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J. A. VAN PARADIJS
G. HAMMERSCHLAG-HENSBERGE
E. P. J. VAN DEN HEUVEL
R. J. TAKENS
E. J. ZUIDERWIJK

Astronomical Institute,
University of Amsterdam,
Roetersstraat 15, Amsterdam,
The Netherlands, and
European Southern Observatory,

C. DE LOORE

Astrophysical Institute,
Free University of Brussels,
Adolph Buyllaan 105,
Brussels, Belgium, and
European Southern Observatory,
La Silla, Chile

Received October 10, 1975; accepted January 13, 1976.

- Hiltner, W. A., Werner, J., and Osmer, P., *Astrophys. J. Lett.*, **175**, L19–22 (1972).
- Jones, C., and Liller, W., *Astrophys. J. Lett.*, **184**, L121–122 (1973).
- Rappaport, S., and McClintock, J., *IAU Circ. No.* 2794 (1975).
- Rappaport, S., and McClintock, J., *IAU Circ. No.* 2833 (1975).
- Zuiderwijk, E. J., van den Heuvel, E. P. J., and Hensberge, G., *Astr. Astrophys.*, **35**, 353–360 (1974).
- Hutchings, J. B., *Astrophys. J.*, **192**, 685–689 (1974).
- Wallerstein, G., *Astrophys. J.*, **194**, 451–457 (1974).
- Wolfe, R. H., Jr., Horak, H. G., and Storer, N. W., in *Modern Astrophysics* (edit. by Hack, M.), 251–273 (Gauthiers-Villars, Paris, 1967).

- ⁹ Avni, Y., and Bahcall, J. N., *Astrophys. J. Lett.* (in the press).
¹⁰ van den Heuvel, E. P. J., in *Astrophysics and Gravitation, Proc. 16th Solvay Conf. Physics*, 119–130 (Brussels University Press, 1974).
¹¹ Hamada, T., and Salpeter, E. E., *Astrophys. J.*, **134**, 683–698 (1961).
¹² Cameron, A. G. W., and Canuto, V., in *Astrophysics and Gravitation, Proceedings of the 16th Solvay Conference on Physics*, 221–267 (Brussels University Press, 1974).
¹³ Blaauw, A., in *Basic Astronomical Data* (edit. by Strand, K. A.), 383–420 (University of Chicago Press, Chicago and London, 1963).
¹⁴ Keenan, P. C., in *Basic Astronomical Data* (edit. by Strand, K. A.), 78–122 (University of Chicago Press, Chicago and London, 1963).
¹⁵ Morton, D. C., and Adams, T. F., *Astrophys. J.*, **151**, 611–621 (1968).
¹⁶ Schlesinger, B., *Astrophys. J.*, **157**, 533–544 (1969).
¹⁷ Osmer, P., *Astrophys. J.*, **181**, 327–348 (1973).
¹⁸ Auer, L. H., and Mihalas, D., *Astrophys. J. Suppl.*, **24**, 193–246 (1972).
¹⁹ Simpson, E. E., *Astrophys. J.*, **165**, 295–316 (1971).
²⁰ Stothers, R., *Astrophys. J.*, **175**, 431–452 (1972).
²¹ Trimble, V., Rose, W. K., and Weber, J., *Mon. Not. R. astr. Soc.*, **162**, 1P–3P (1973).

Implications from the absence of a ^{41}K anomaly in an Allende inclusion

CLAYTON¹ has elaborated on his unorthodox interpretation² of the occurrence in meteorites of the decay products of the extinct radionuclides ^{129}I and ^{244}Pu . He has suggested that these radioactive species did not decay in the meteorites but rather in interstellar grains which, on the formation of the meteorites, were incorporated without much alteration. Thus, the concentration in the meteorites of the decay products does not reflect the time of meteorite formation relative to the cessation of nucleosynthesis but is essentially governed by the fraction of interstellar grains mixed with the meteoritic matter proper. If that is true, anomalies will occur in the isotopic composition of numerous elements. The decisive criterion of whether an over-abundance of a given isotope can be expected is the production yield during nucleosynthesis of a radioactive precursor; the half life of that precursor is of only minor importance.

Among the possible anomalies mentioned by Clayton is an excess of ^{41}K . According to Woosley *et al.*³ this nuclide is synthesised predominantly as ^{41}Ca , its radioactive progenitor. Therefore, in an early condensate from the solar nebula, enriched in Ca relative to K, ^{41}K should be over-abundant provided that no homogenisation of potassium has occurred later on. We wish to report on a potassium isotopic analysis performed on an inclusion from the Allende carbonaceous chondrite.

For three reasons Allende seems to be a promising meteorite. First, it is almost unanimously agreed that it contains inclusions of an early primary condensate from the solar nebula (for an alternative view see ref. 4). Second, variations in the isotopic composition of oxygen⁵ and magnesium^{6,7} in the inclusions show that a disequilibrium distribution has been frozen in and indicate that those two elements at least have not been homogenised isotopically during the subsequent history of the meteorite. Third, almost by definition, Ca, being a refractory element, is enriched in such inclusions, whereas potassium is depleted. The resultant Ca/K ratio is thus exceedingly high, which results in a high sensitivity for the detection of a possible contribution from ^{41}Ca to ^{41}K .

We conducted our experiment with a 10.6-mg sample of a white chondrule previously analysed by thermal neutron activation analysis⁸. (Although in the course of this analysis the sample was exposed to a dose of 1.5×10^{16} thermal neutrons cm^{-2} , it is of no consequence to the investigation.) After the sample was dissolved the potassium was separated on Dowex 50 WX8 and analysed by mass spectrometry. Details of the chemical procedure and the isotopic analysis have been given elsewhere⁹. Results pertinent to this discussion are—K-content: 210 ± 20 p.p.m.; Ca/K: 860 ± 100 (see ref. 8); potassium blank: 3.4 ± 0.5 μg ; measured ratios: $^{39}\text{K}/^{41}\text{K} = 13.997 \pm 0.015$; $^{41}\text{K}/^{40}\text{K} = 575.3 \pm 3.7$.

As we made no attempt to determine absolute isotope abundances the measured ratios have to be normalised. We therefore analysed a terrestrial potassium standard. Using the NIER (ref. 10) value of $^{39}\text{K}/^{41}\text{K} = 13.47$, and assuming the mass discrimination between neighbouring isotopes to be constant, we obtained $^{41}\text{K}/^{40}\text{K} = 584.87 \pm 0.8$ (mean value of

16 runs) which is in good agreement with that of 584.89 given by Burnett *et al.*¹¹ who normalised to the same $^{39}\text{K}/^{41}\text{K}$ ratio.

Because it is necessary to normalise the measured ratios an unequivocal analysis of the Allende data is only possible if any potential anomaly occurs in the abundance of one isotope only. Thus, assuming that in the Allende inclusion the $^{39}\text{K}/^{40}\text{K}$ ratio is identical to the terrestrial value our result shows that the enrichment in the abundance of ^{41}K is $\delta^{41}\text{K} = (0.55 \pm 1.3)\%$. If the $^{39}\text{K}/^{41}\text{K}$ ratios are the same in both cases $\delta^{40}\text{K} = -(0.3 \pm 0.7)\%$. It is worth mentioning that, within reasonable limits, the δ values are rather insensitive to the ratios used for normalisation. A change in the choice for the terrestrial $^{39}\text{K}/^{41}\text{K}$ by $\pm 5\%$ results in even smaller, but comparable, relative changes of the δ values.

Within the stated errors the isotopic composition of potassium from the Allende inclusion is indistinguishable from that of terrestrial potassium. This constrains certain interesting parameters. The absolute excess in the abundance of ^{41}K is $\Delta^{41}\text{K} = [\text{K}] \times \text{H} (^{41}\text{K}) \times \delta^{41}\text{K}$ where $[\text{K}]$ is the potassium content (including blank) and $\text{H} (^{41}\text{K})$ the isotopic abundance of ^{41}K . This is equal to the amount of ^{41}Ca ($= ^{41}\text{Ca}/^{40}\text{Ca} \times [\text{Ca}] \times \text{H} (^{40}\text{Ca}) \times f$) which decayed in the sample. Assuming^{1,2} that the condensation of Ca took place quickly after the synthesis of ^{41}Ca , that is, that no correction has to be applied for its decay (half life 1.3×10^5 yr) before condensation (see refs 1 and 2), f denotes the fraction of presolar Ca in the sample. The data presented here show that, on the 3σ level ($\delta^{41}\text{K} \leq 5\%$), $^{41}\text{Ca}/^{40}\text{Ca} \cdot f \leq 10^{-5}$. Clayton² reasons that for refractory elements like Ca, $f \geq 0.1$. Thus, in the presolar grains at the time of their formation the $^{41}\text{Ca}/^{40}\text{Ca}$ ratio must have been $\leq 10^{-4}$. According to Peters *et al.*¹², however, the production ratio in explosive carbon, oxygen, or silicon burning is about a factor of 30 higher (1/320).

Of the numerous possibilities which could account for the discrepancy between our experimental result and theoretical prediction a few are worth listing.

- The cross sections relevant to the $^{41}\text{Ca}/^{40}\text{Ca}$ production ratio are not known; they could be considerably lower than assumed¹³.

- Even for refractory elements the fraction, f , of presolar grains in the meteoritic matter may be lower than 0.1, although it is doubtful that with a 30-fold reduction the ^{244}Pu data are still reconcilable with the model.

- Radioactive decay of ^{41}Ca between cessation of nucleosynthesis and condensation of the grains may not be negligible after all; a free decay interval of about 0.5 Myr would be sufficient. In that case the number of possible isotope anomalies mentioned by Clayton¹ will be drastically reduced, as ^{41}Ca has one of the longest half lives among the radioactive progenitors.

- Homogenisation of potassium and/or calcium may have occurred in the sample analysed. This would have wiped out, or drastically reduced, any anomaly which may have existed. A late addition of potassium of normal isotopic composition is not sufficient, however.

- The isotope anomaly in potassium may not be restricted to one isotope only, as has had to be assumed here for an unambiguous interpretation. Only a determination of the absolute abundance ratios without normalisation will allow that possibility to be checked.

As we realise that it would be helpful to know whether our sample shows anomalies in the isotopic composition of other elements, such as Mg, such measurements are planned.

F. BEGEMANN
W. STEGMANN

Max-Planck-Institut für Chemie,
(Otto-Hahn-Institut),
65 Mainz, W. Germany

Received November 19, 1975; accepted January 2, 1976.

¹ Clayton, D. D., *Nature*, **257**, 36 (1975).

² Clayton, D. D., *Astrophys. J.*, **199**, 765 (1975).

³ Woosley, S. E., Arnett, W. D., and Clayton, D. D., *Astrophys. J. Supp. Ser.*, **26**, 231 (1973).

- ⁴ Blander, M., and Fuchs, L. H., *Geochim. cosmochim. Acta* (in the press).
⁵ Clayton, R. N., Grossman, L., and Mayeda, T. K., *Science*, **182**, 485 (1973).
⁶ Gray, C. M., and Compston, W., *Nature*, **251**, 495 (1974).
⁷ Papanastassiou, D. A., and Lee, T., *J. geophys. Res. Lett.*, **1**, 225 (1974).
⁸ Wanke, H., Baddenhausen, H., Palme, H., and Spettel, B., *Earth planet. Sci. Lett.*, **23**, 1 (1974).
⁹ Stegmann, W., and Begemann, F., *Z. Naturf.*, **30a**, 968 (1975).
¹⁰ Nier, A. O., *Phys. Rev.*, **77**, 789 (1950).
¹¹ Burnett, D. S., Lippolt, H. J., and Wasserburg, G. J., *J. geophys. Res.*, **71**, 1249 (1966).
¹² Peters, J. G., Fowler, W. A., and Clayton, D. D., *Astrophys. J.*, **173**, 637 (1972).

Devonian palaeogeography of the Orcadian Basin and the Great Glen Fault

STORETVEDT¹ has argued that Devonian palaeomagnetic results from Norway and northern Scotland indicate a post-Devonian sinistral displacement along the Great Glen Fault of some 200–300 km. This has been objected to on geological grounds by Mykura² and on palaeomagnetic grounds by

Turner *et al.*³. Here we present a simplistic Devonian palaeogeographical model of the Orcadian Basin based on observation of onshore sediments. We believe the model is compatible with only minor dextral displacement along the Great Glen Fault in post-Devonian times.

A variety of modes of displacement have been put forward for the Great Glen Fault. Some are based on pre-Devonian evidence^{4,5} and are not considered relevant to our argument. We consider the following three:

First, sinistral movements of 200–300 km (ref. 1) or ~ 500 km (ref. 6).

Second, dextral movements of:

- (1) ~ 30 km on the Great Glen Fault *s.s.*⁷
- (2) ~ 65–80 km on the Walls Boundary Fault in Shetland^{8–11}
- (3) a considerable movement on the Melby Fault in Shetland¹²

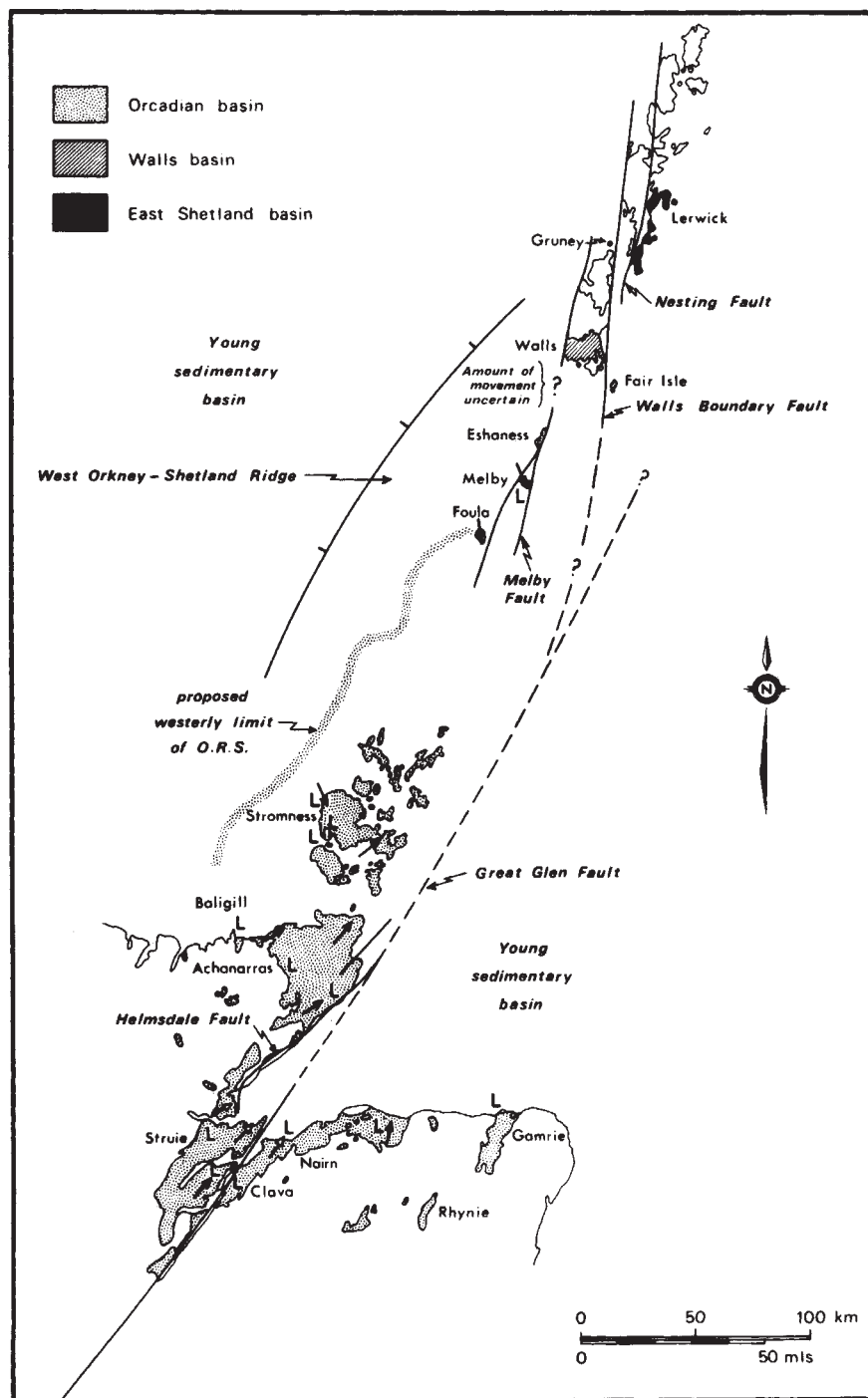


Fig. 1 Reconstruction of Devonian palaeogeography in the Orcadian Basin. Sources as described in the text, plus Watts²¹. L is the Achanarras limestone and equivalents. Open arrows show Upper ORS palaeocurrents (RA) and closed arrows show Middle ORS palaeocurrents (Orkney from Mykura¹², elsewhere from R.N.D.).

(4) ~ 50 km on the Helmsdale Fault⁸

(5) a substantial movement on the Latheron Fault in south eastern Caithness¹³.

Third, normal movements:

(1) Post-Jurassic movement of the Great Glen Fault s.s. associated with development of the Moray Firth Basin¹⁴

(2) Post Jurassic movement of the Walls Boundary Fault associated with basin development to the south west of Shetland¹⁵.

Of the three distinct basins of ORS sedimentation in Shetland, considered to be juxtaposed by dextral faulting^{2,12} only the westernmost had tangible connection with the Orcadian Basin to the south (Fig 1), that is, the similarity of the fauna of the Melby Fish Beds with those at Sandwick (Orkney) and Achanarras (Caithness). Mykura¹² has shown that a post-Devonian sinistral movement of 200–300 km is unlikely, as it places the dissimilar Orcadian and East Shetland sequences in juxtaposition. A movement of ~ 500 km certainly resolves the contradiction but has the great disadvantage of placing the ORS sediments of the southern Basin to the east of the Great Glen Fault system, in a position now occupied by the coast of Galway, western Ireland. Such a position is unlikely as the ORS sediments of the Orcadian Basin on both sides of the Great Glen Fault are very similar in character and show an identical history of development which is unique in the British Isles, and comprises:

- (1) Lower ORS (Upper Seigennian and Emsian) sedimentation of mainly alluvial character (ref. 16 and A. Collins, R.N.D. and R.A., unpublished).
- (2) Unconformity associated with mild earth movements.
- (3) Middle ORS (Eifelian and Givetian) sedimentation of mixed lacustrine and alluvial character.
- (4) Unconformity associated with earth movement and volcanism in Orkney.
- (5) Upper ORS (Frasnian and Lower Fammenian) sedimentation of alluvial character¹⁵.

During the extensive Middle ORS deposition faunal evidence indicates a most intimate connection between areas on either side of the Great Glen Fault system. Specifically the Achanarras Fish Bed fauna is represented in the southern part of the Orcadian Basin at, for example, Edderton and Cromarty to the west of the fault system and Clava, Nairn, Lethen Bar, Tynet Burn and Gamrie to the east (Fig. 1). The age equivalence of these beds is based on vertebrate faunas^{17,18} and also on palynological data¹⁹. Furthermore, the zonal faunal assemblages recognised in the Caithness Flagstones¹³ occur to the east of the Great Glen Fault system in the Nairn–Clava region in identical stratigraphic order²⁰. At least 18 species of fish are, in fact, common to both sides of the fault system.

The existing outcrops of the Middle ORS are remnants of the western and southern margins of an elliptical basin elongated in a north-east–south-west direction (Fig. 1). The original focus of sedimentation in this basin (as exposed) was in the Caithness–Orkney area where a distinctive, dominantly lacustrine, facies developed. Subsequently overlap to the north, west, south and south-east took place. In these latter areas alluvial deposits are more in evidence. Throughout the basin a rhythmic pattern of sedimentation was produced by variation in the level of Orcadian lakes. During high lake levels, when lake waters lapped directly against the underlying Caledonian basement, a distinctive marginal limestone facies was deposited^{21,22}. This facies has recently been found on the south shore of the Moray Firth and confirms the overlap discussed above. Palaeocurrent vectors in both the Middle and Upper ORS support deposition of the sediments within a single basin (Fig. 1).

Within the palaeogeographic framework a relatively modest post-Devonian dextral shift of 30 km (ref. 7) is

acceptable and indeed produces a better 'fit' of Middle ORS outcrops in the Inverness area. There would seem to be no geological evidence to support large scale post-Devonian sinistral movement along the Great Glen Fault system although a variety of motions could have occurred in pre-Devonian times.

R. N. DONOVAN

Department of Geology,
Oklahoma State University,
Stillwater, Oklahoma 74074

R. ARCHER

Department of Geology,

P. TURNER

D. H. TARLING

Department of Geophysics and Planetary Physics,
The University,
Newcastle upon Tyne NE1 7RU, UK

Received July 7; accepted December 18, 1975.

- ¹ Storetvedt, K. M., *Geol. Mag.*, **111**, 23–30 (1974).
- ² Mykura, W., *Geol. Mag.*, **112**, 91–94 (1975).
- ³ Turner, P., Tarling, D. H., and Donovan, R. N., *Geol. Mag.* (in the press).
- ⁴ Garson, M. S., and Plant, J., *Nature*, **240**, 31–35 (1972).
- ⁵ Winchester, J. A., *Nature phys. Sci.*, **246**, 81–83 (1973).
- ⁶ Storetvedt, K. M., *Geol. Mag.*, **112**, 94–96 (1975).
- ⁷ Holgate, N., *Scott. J. Geol.*, **5**, 97–139 (1969).
- ⁸ Flinn, D., *Geol. J.*, **6**, 272–292 (1969).
- ⁹ Mykura, W., and Young, B. R., *Report No. 69/4, Inst. geol. Sci.* (1969).
- ¹⁰ Mykura, W., *Bull. geol. Surv. Gt. Br.*, **41**, 1–31 (1972).
- ¹¹ Mykura, W., *Bull. geol. Surv. Gt. Br.*, **41**, 33–53 (1972).
- ¹² Mykura, W., *J.G.S. Regional Guide* (in the press).
- ¹³ Donovan, R. N., Foster, R. J., and Westoll, T. S., *Trans. R. Soc. Edinb.*, **69**, 167–201 (1974).
- ¹⁴ Bacon, M., and Cheshire, J. A., *Scott. J. Geol.*, **11** (in the press).
- ¹⁵ Bott, M. H. P., and Browitt, C. W. A., *J. geol. Soc. Lond.*, **131**, 353–371 (1975).
- ¹⁶ Richardson, J. B., *Rev. Palaeobot. Palynol.*, **1**, 111–129 (1967).
- ¹⁷ Westoll, T. S., *Int. Geol. Congress 1948*, xi, 5–20 (1951).
- ¹⁸ Miles, R. S., and Westoll, T. S., *Trans. R. Soc. Edinb.*, **65**, 179–213 (1963).
- ¹⁹ Richardson, J. B., *Palaeontology*, **5**, 171–194 (1962).
- ²⁰ Horne, J., *Mem. geol. Survey Gt. Brit.*, **128** pp. (1923).
- ²¹ Donovan, R. N., *Scott. J. Geol.*, **9**, 203–211 (1975).
- ²² Donovan, R. N., *J. geol. Soc. Lond.* (in the press).
- ²³ Watts, A. B., *Scott. J. Geol.*, **7**, 189–218 (1971).

Kinetic processes and thermal history of slowly cooling solids

IN solids, the rates of kinetic processes such as volume diffusion and order-disorder transitions are extremely sensitive to temperature. At room temperatures they are so slow that crystalline solids normally preserve a substantial degree of thermodynamic disequilibrium over very long periods (> 10⁹ yr in some cases). At sufficiently high temperatures, on the other hand, some processes go so fast that a continuously varying state of equilibrium may be maintained as the temperature changes. When a solid cools steadily a transition occurs from the one state (continuous equilibrium) to the other (false equilibrium) in the neighbourhood of the closure temperature, which is defined below. Here I present a simple quantitative argument relating closure temperature to the cooling rate and kinetic parameters of a given kinetic system (see refs 1–6 for related work).

Closure temperature¹ is defined in Fig. 1. Curve I represents the equilibrium value of some chemical parameter x , and curve II its kinetically-controlled value. At high temperatures the two curves coincide. When the temperature decreases sufficiently the kinetically-controlled value begins to change more slowly than the equilibrium value, and the curves diverge. Eventually x reaches a constant, non-equilibrium value, corresponding to some earlier temperature (see Fig. 1), which is the closure temperature T_c . Slower rates of cooling allow more time for re-equilibration, and therefore lead to lower values of T_c .

Analytic derivations of closure temperature have been presented for some simple first-order kinetic systems¹. A graphical solution has been attempted for one second-order system³. Here a physical argument is given which is applicable, in an approximate way, to all kinetic systems which obey the Arrhenius law. The argument compares a cooling time constant

with estimated isothermal reaction times at various temperatures.

For Arrhenius processes the reaction rate coefficient $k(T)$ at absolute temperature T is given by

$$k(T) = k_0 \exp(-E/RT) \quad (1)$$

E is the activation energy and k_0 the frequency factor for the process, and R is the gas constant. For a slowly cooling system

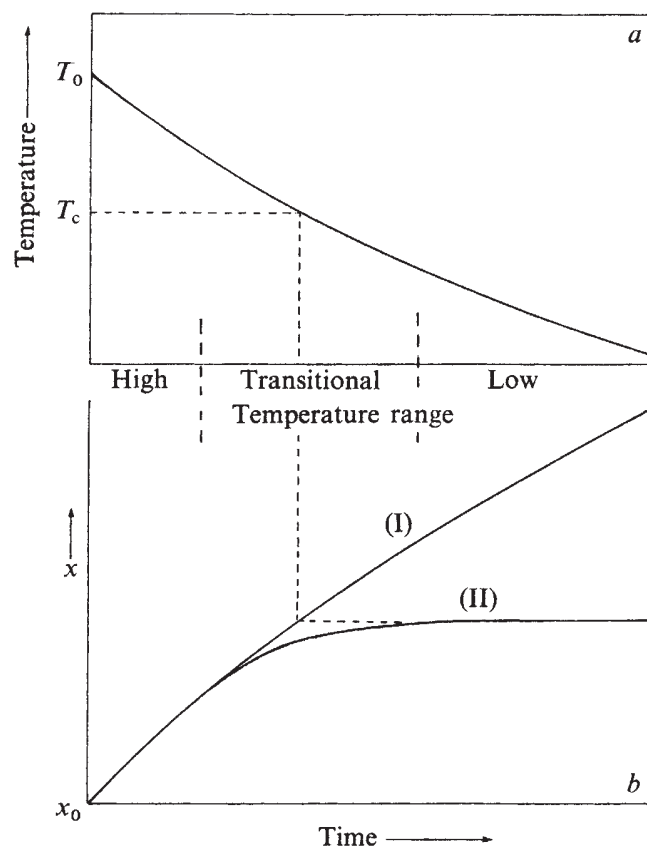


Fig. 1 Kinetically controlled closure in cooling solids. *a*, Temperature against time; *b*, parameter x against time. (I), Equilibrium value of x ; (II), actual value of x .

k diminishes roughly exponentially with time. If we make the approximation

$$T^{-1} = T_0^{-1} + \text{constant} \times t \quad (2)$$

Multiplying by E/R we absorb the above constant in the cooling time constant τ

$$E/RT = E/RT_0 + t/\tau \quad (3)$$

Substituting in (1) we obtain

$$k(t) = k(T_0) \exp(-t/\tau) \quad (4)$$

so that τ is the time required for k to diminish by a factor e in the neighbourhood of T . The relationship between τ and the cooling rate $\dot{T}$ is found by differentiating (3) to be

$$\tau = -RT^2/E\dot{T} \quad (5)$$

We can now compare τ with the time required for the kinetic process to go, say, half-way towards completion at constant

temperature, which must be inversely related to the rate coefficient, so we can write

$$kt_{1/2} = \alpha \quad (6)$$

(For a first-order process $\alpha = 0.693$; for simple diffusion processes it is smaller and depends on the initial distribution and the geometry; for second or higher-order kinetics α depends to some extent on both the actual value of x and its equilibrium value.) At high temperatures the process must reach equilibrium in a time which is short compared with the time required for k to diminish significantly, so we infer that $t_{1/2} \ll \alpha$. At low temperatures, on the other hand $t_{1/2} \gg \alpha$: that is to say, the process is so slow that negligible progress is made towards equilibrium before the rates constant has diminished to even lower value. Finally, in the transitional range, where the temperature cools through the value T_c , $t_{1/2}$ and τ are of the same order of magnitude. Thus from (6) we have

$$\tau k(T_c) \simeq \alpha \quad (7)$$

Substituting for $k(T_c)$ and taking logarithms we obtain immediately

$$E/RT_c \simeq \ln(\tau k_0/\alpha) \quad (8)$$

Analytical solutions for first-order kinetics and for simple diffusion processes¹, give

$$E/RT_c = \ln(A\tau k_0) \quad (9)$$

in which k_0 may be replaced by D_0/a^2 , the frequency factor for a diffusing component in a system with characteristic dimension a . Thus it seems that equation (9) can be generalised to more complex kinetics by setting $A \simeq \alpha^{-1} = (kt_{1/2})^{-1}$. Assuming the boundary condition (curve I) to be linear in $1/T$ over the transitional temperature range, and therefore a linear function of time, the constant A is 1.8 for first-order reactions, and 8.7, 27 and 55 for diffusion out of a plane slab, cylinder or sphere¹ comparison with equation (6) and (8) using data from Crank⁷ shows that, in these systems, a better approximation to A is given by

$$A = 2/kt_{1/2} = 2/\alpha \quad (10)$$

This gives values of A which are too high by 60% for a first-order reaction, but only 20% for diffusion problems.

Eliminating τ between equations (5) and (9), and setting $T = T_c$, we obtain

$$E/RT_c = \ln(-Ak_0RT_c/2E\dot{T}) \quad (11)$$

which can be solved explicitly for $\dot{T}$, or iteratively for T_c (k_0 can be replaced by D_0/a^2 when appropriate). The relationship between T_c and $\dot{T}$ is plotted in Fig. 2 for various values of activation energy E . Because of the logarithmic character of the equation, T_c is rather insensitive to cooling rate, especially at low temperatures and high activation energies. For example, at 60 kcalorie mol⁻¹ a change in the cooling rate by a factor of ten changes T_c by only 20° near 250°C, or 80° near 1,000°C. At 10 kcalorie mol⁻¹ the corresponding changes are 100° and 400°. The corollary is that observations of closure temperature must be very precise to yield useful information about cooling history. The best that has yet been achieved is represented by a study of nickel concentration profiles in iron meteorites, in which numerical modelling of the diffusion/cooling process led to uncertainties of $\pm 15\%$ – $\pm 50\%$ in the estimated cooling rates⁸. On the other hand, an approximate knowledge of cooling rates may yield rather accurate predictions of closure temperature, which may be helpful in understanding how the properties of materials depend on their cooling history (see, for example, ref. 6).

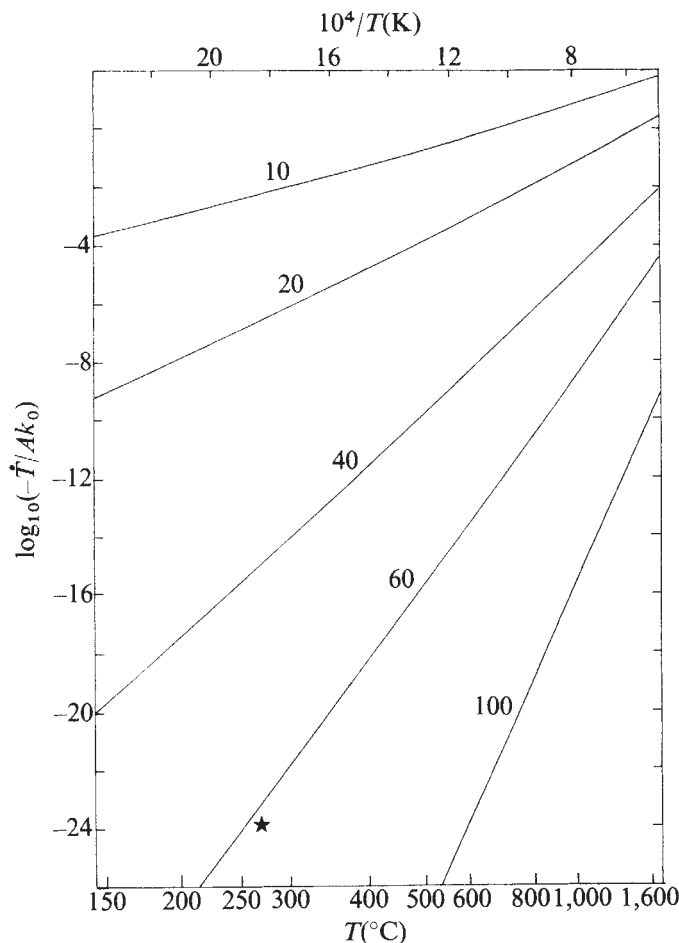


Fig. 2 Relationship between closure temperature T_c , cooling rate $\dot{T}$, and kinetic parameters. Figures on curves denote values of activation energy E in kcal/mol. Star corresponds to example discussed in text.

As an example for which analytic solutions are not yet available, we may consider the Fe^{2+} -Mg order-disorder reaction in a cooling anthophyllite³. A closure temperature of 270 °C was estimated for the untreated sample, while laboratory kinetic studies⁹ gave $E = 61.6$ kcal/mol and $k_0 = 8.6 \times 10^{17} \text{ yr}^{-1}$. Inspection of the kinetic data⁹ yields an estimate for A of ~ 10 , from which, using equation (11) or Fig. 2 one can calculate a cooling rate of 13 °C per million years. Seifert and Virgo³ estimated, graphically, a maximum cooling rate of 100 °C per million years for this material, while typical cooling rates in Alpine metamorphic rocks are 20 °C Myr⁻¹ (refs 1, 10 and 11). The principal weakness in the calculation is uncertainty in A , since for a second-order reaction, $t_{1/2}$ depends on the initial concentrations. In such cases, the best estimate of A (other than that obtained from a full analytical or numerical solution to the problem) could perhaps be obtained by estimating $kt_{1/2}$ for an isothermal reaction which starts with a degree of disequilibrium comparable with that expected within the transition temperature range of the cooling system.

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M. H. DODSON*

Department of Terrestrial Magnetism,
Carnegie Institution of Washington,
5241 Broad Branch Road, N.W.,
Washington, DC 20015

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*Present address: Department of Earth Sciences, University of Leeds, Leeds LS2 9JT, UK.

¹ Dodson, M. H., *Contr. Miner. Petrol.*, **40**, 259–276 (1973).

² Wagner, G. A., and Reimer, G. M., *Earth planet. Sci. Lett.*, **14**, 263–268 (1972).

- ³ Seifert, F., and Virgo, D., *Science*, **188**, 1107–1109 (1975).
⁴ Lasaga, A. C., and Richardson, S., *EOS, Trans. Am. Geophys. Un.*, **56**, 459 (1975).
⁵ Sykes, C., and Evans, H., *J. Inst. Metals*, **58**, 255–280 (1936).
⁶ Burke, J., and Stuckey, D., *Phil. Mag.*, **31**, 1063–1080 (1975).
⁷ Crank, J., *Mathematics of Diffusion* (Oxford, Clarendon Press, 1956).
⁸ Goldstein, J. I., and Short, J. M., *Geochim. cosmochim. Acta*, **31**, 1001–1023 (1967).
⁹ Seifert, F., and Virgo, D., *Yb. Carnegie Inst. Wash.*, **73**, 405–411 (1974).
¹⁰ Jäger, E., Niggli, E., and Wenk, E., *Beitr. geol. Karte Schweiz*, N.F. 134 (1967).
¹¹ Clark, S. P., and Jäger, E., *Am. J. Sci.*, **267**, 1143–1160 (1969).

Thermal conductivity of allotropic modifications of ice

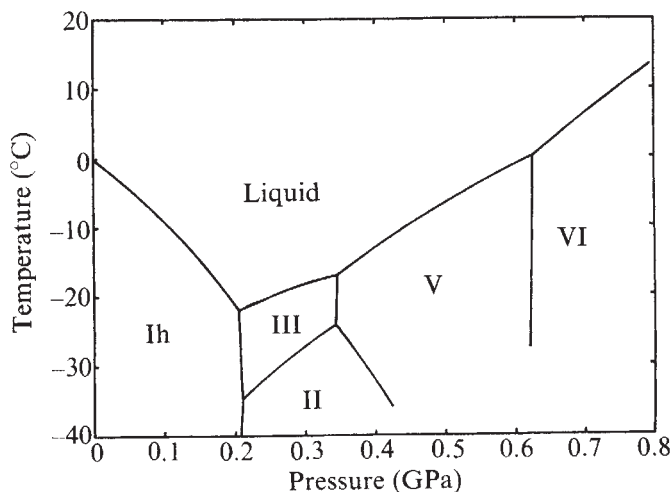
THE pressure-temperature phase diagram of H_2O comprises several solid phases, differing in their crystal structure (Fig. 1)¹. Ice offers an unusual opportunity for investigating how the different modes of stacking and the slight distortions of otherwise identical molecules influence the conduction of heat. There are no previous studies of the thermal conductivity of ice, except for the phase Ih at atmospheric pressure. We report here the first measurements of the thermal conductivity of the high pressure forms II, III, V and VI. Each of these modifications has a higher density, and a lower thermal conductivity, than ordinary ice, Ih. Ice Ih seems to be the first example of an insulator which has a decreasing thermal conductivity under increasing pressure. Our findings show that all these forms of ice have a positive thermal expansion coefficient.

Our measurements were carried out using the transient hot-wire method², in which a pulse of constant electrical power is supplied to a metal wire immersed in a medium of the substance under study. An analysis of the change of temperature over time in the wire, as monitored by its electrical resistance, enables the thermal conductivity to be determined. The pulse length used was 0.6 s. Temperature data were acquired and reduced using a programmable desk calculator. We used a nickel wire, 0.1 mm in diameter, 40 mm long between the potential leads.

The wire was situated across the diameter of a Teflon cell similar to that described by Andersson and Bäckström³, but 70 mm in diameter. This cell was filled with doubly distilled H_2O , which was degassed by boiling. The cell was subjected to pressure in a piston-cylinder apparatus, cooled by circulating Freon.

Our measurements were made over a range of pressures going up to 0.65 GPa, and at temperatures down to -35 °C. Steady-state temperature measurements were taken using three thermocouples with their junctions located at different radial distances from the axis of the vessel. Phase transitions were detected using some or all of the following observations: discontinuous changes in the thermal conductivity, λ ; thermocouple responses indicating latent heat; and piston displacements indicating changes of volume. Whenever the measurements showed clearly that the system was fully within a single phase

Fig. 1 Phase diagram of H_2O .



region, the temperature differences within the cell were less than 0.2 °C. We were careful to ensure that temperature excursions of the Ni wire did not raise the temperature of the ice above the range of the modification under investigation.

Our investigations of the thermal expansion coefficient, α , have revealed that an approximately adiabatic pressure increase or decrease always results in a temperature change of the same sign. Using the identity $(\partial T/\partial P)_s = T\alpha/c_p$, we conclude that α is positive in the pressure-temperature region investigated, whereas it is known to have negative values for water and, according to one report¹, for ice Ih at very low temperatures.

Our results for λ for each of the phases of ice are given in Table 1. Data for liquid H₂O (see ref. 4) are quoted for comparison. Special efforts were not made to attain an absolute degree of accuracy, but our average value for ice Ih at -25 °C and atmospheric pressure— $\lambda = 2.38 \text{ W m}^{-1} \text{ K}^{-1}$ —is within the limits of error of other determinations^{5,6}. We measured the conductivity of the five solid phases several times and in different orders in pressure-temperature space, and the results agreed to within 1 or 2% in each case. The temperature variation of the conductivity in phase Ih was found to conform to the $1/T$ dependence already reported^{5,6}. But a startling fact emerged concerning the pressure dependence. We found that the thermal conductivity decreases by 6% in going from atmospheric pressure to 0.20 GPa, whereas all the insulators previously investigated have exhibited an increase^{7,8}. In the other phases of ice no temperature or pressure dependence could be detected over the temperature-pressure region investigated.

A rough estimate of the phonon mean free path, L (some values of L are included in Table 1), may be obtained from the well known Debye formula

$$\lambda = c_p L / 3(\nu\kappa)^{1/2}$$

In calculating L we have taken the specific volumes, v , from

Table 1 Thermal conductivity of H₂O

Phase	T (°C)	P (GPa)	λ (W m ⁻¹ K ⁻¹)	L (nm)
Liquid	0	0.00	0.56	0.29
Ih	-25	0.00	2.38	1.20
II	-30	0.30	1.65	
III	-25	0.25	0.98	0.43
V	-25	0.50	1.24	0.44
VI	-20	0.65	1.54	0.60

Bridgman⁹ and used his isothermal compressibilities except for ice Ih (see ref. 10) and liquid (see ref. 11), for which the adiabatic compressibilities are known. Furthermore, we have assumed the specific heat capacity to be the same in all the solid phases. The point to be noted is that, for phases III, V and VI, L is on average about twice the value that it is for the liquid, whereas for ice Ih it is four times as large.

Bridgman⁹ has provided data for phase Ih, showing a rapid decrease of compressibility with pressure, which would lead to a similar decrease of L . But his atmospheric pressure value for compressibility is at variance with later work¹². Thus, although it would be interesting to calculate the pressure dependence of L for ice Ih, we feel this cannot yet be done reliably.

We have considered the possibility that a single crystal of ice Ih present initially near atmospheric pressure would gradually transform into a polycrystalline mass, resulting in an increased grain scattering. Measuring λ for decreasing as well as increasing pressure, however, we obtained perfectly reproducible results, from which we conclude that changes in grain boundary scattering cannot be invoked to explain the pressure dependence.

The phonon mean free path, L , cannot be similarly computed for ice II, since the compressibility is unknown. It should be noted, however, that the thermal conductivity of this phase is clearly larger than for other phases with a comparable density. We tentatively attribute this to the proton order in the

structure of ice II, which does not exist in the other phases. We hope to shed light on the influence of proton order by making similar measurements on D₂O and its mixtures with H₂O.

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R. G. ROSS
P. ANDERSSON
G. BÄCKSTRÖM

Department of Physics,
Umeå University,
S-901 87 Umeå, Sweden

Received December 15, 1975; accepted January 5, 1976.

- Hobbs, P. V., *Ice Physics* (Oxford University Press, Oxford, 1974).
- Andersson, P., and Bäckström, G., *Rev. scient. Instrum.* (in the press).
- Andersson, P., and Bäckström, G., *Rev. scient. Instrum.*, **46**, 1292-1293 (1975).
- Powell, R. W., *Adv. Phys.*, **7**, 276-297 (1958).
- Ratcliffe, E. H., *Phil. Mag.*, **7**, 1197-1203 (1962).
- Dillard, D. S., and Timmerhaus, K. D., *Bull. Inst. Int. Froid.*, **Suppl. 2**, 35-44 (1966).
- Alm, O., and Bäckström, G., *J. Phys. Chem. Solids*, **35**, 421-424 (1974).
- Andersson, P., and Sundqvist, B., *J. polym. Sci., Pt A-2*, **13**, 243-251 (1975).
- Bridgman, P. W., *Proc. Am. Acad. Arts Sci.*, **47**, 441-558 (1911).
- Dantli, G., in *Physics of Ice* (edit. by Richl, N., Bullemer, B., and Engelhardt, H.), 223-230 (Plenum, New York, 1969).
- Kell, G. S., in *Water: A Comprehensive Treatise*, **1** (edit. by Franks, F.), 363-412 (Plenum, New York, 1972).
- Gow, A. J., and Williamson, T. C., *J. geophys. Res.*, **77**, 6348-6352 (1972).

Intensity effects in the electrochemical photolysis of water at the TiO₂ electrode

FUJISHIMA and Honda¹ have described the photosensitised electrolysis of water on n-type TiO₂ single-crystal electrodes and proposed this reaction be used for solar-energy conversion. The process differs from conventional electrolysis since photocurrents can be generated without the application of an external potential, and thus direct conversion of light to electrical energy is possible. Since their report, several groups have investigated this system²⁻⁴ but, as a consequence of the broad-band irradiation used, accurate quantum yields could not be determined. Here we report successful measurements of the variation of quantum efficiency with intensity. Our main finding is that these crystals become efficient converters if a small voltage is applied, even at intensities of 12 mW cm⁻².

This is important since the degree to which sunlight can be focused without decreasing the quantum efficiency will determine whether these devices may be used economically. Intensity effects might be expected in this system since a major factor in determining the magnitude of the steady-state photocurrent is the recombination rate of the photoproduced valence-band holes and conduction-band electrons⁵.

To study these effects, a dual compartment electrochemical cell and single-crystal electrode were made¹. A platinum cathode was used and a PAR-174 polarograph in the circuit allowed us to adjust the potential of the TiO₂ relative to a saturated calomel electrode (SCE). Small dark currents resulted from the application of this potential. When the TiO₂ electrode was illuminated, substantially higher currents were observed, and the difference between the observed current and the dark current was used to calculate the quantum efficiency of the process. The light source was a Coherent Radiation CR-8 argon ion laser emitting a doublet at 351 and 364 nm. The power incident on the electrode was varied through the use of neutral density filters, the resulting photocurrent was monitored, and the existence of a nonlinear intensity dependence was confirmed. The results depicted in Fig. 1 show marked deviation from linearity both at 0 and +2.0 V (SCE).

To obtain more data in the lower intensity range, the laser beam was directed through a beam expander on to the TiO_2 surface. The resulting power loss was $\sim 70\%$, but with the focusing lens of the expander, it was possible to vary the size of the spot of light on the electrode surface from 0.2–2.0 cm in diameter, while maintaining constant total output power. This had the effect of varying intensity per unit area at the electrode. Three solutions having the same electrical conductivity but with different pH were used in the experiments. Results of irradiations performed at 0 V (SCE) are depicted in Fig. 2a. The dashed line represents the photocurrent expected if the quantum yield was unity and was intensity independent. Generation of the photocurrent in all three solutions becomes increasingly less efficient as intensity increases, and marked differences in behaviour are exhibited. The lowest efficiencies occur consistently for the acidic solution and the quantum yield at very low intensities is < 0.1 . With the NaOH solution at intensities $< 10 \text{ mW cm}^{-2}$, the photocurrent is approximately linearly dependent on intensity, and the quantum yield calculated from the slope in this region is 0.7. This behaviour suggests that a pH-dependent process occurs at 0 V (SCE) at the surface of the electrode, and effects electron-hole recombination.

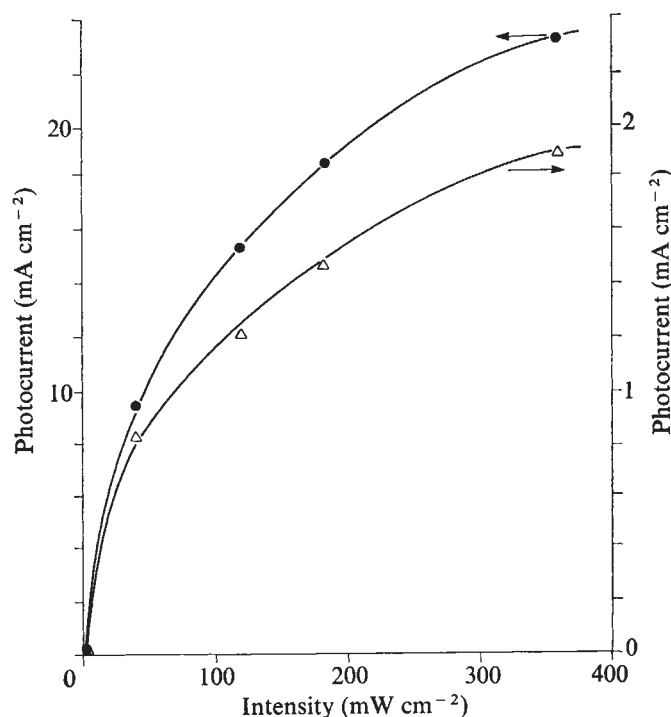


Fig. 1 The photocurrent as a function of intensity for irradiation of the n-TiO_2 electrode in 0.51 N HClO_4 for applied potentials of (Δ , right axis) 0 V (SCE) and ($\bullet$, left axis) +2 V (SCE).

When the potential of the TiO_2 electrode was raised to +1.0 V and the experiments repeated, the situation was substantially altered (Fig. 2b). Above 12 mW cm^{-2} , all three plots deviated from linearity, but even at the greatest intensity examined, the quantum yields were still high. The quantum yield of electrons at 60 mW cm^{-2} in acidic solution was 0.55 whereas in the basic solution it was 0.67. Below 12 mW cm^{-2} , the three curves are coincident and a nearly linear intensity dependence is obtained. The electron quantum yield calculated from the slope in this region is 0.85. For intensities $< 0.5 \text{ mW cm}^{-2}$, we have found quantum yields as high as 0.95.

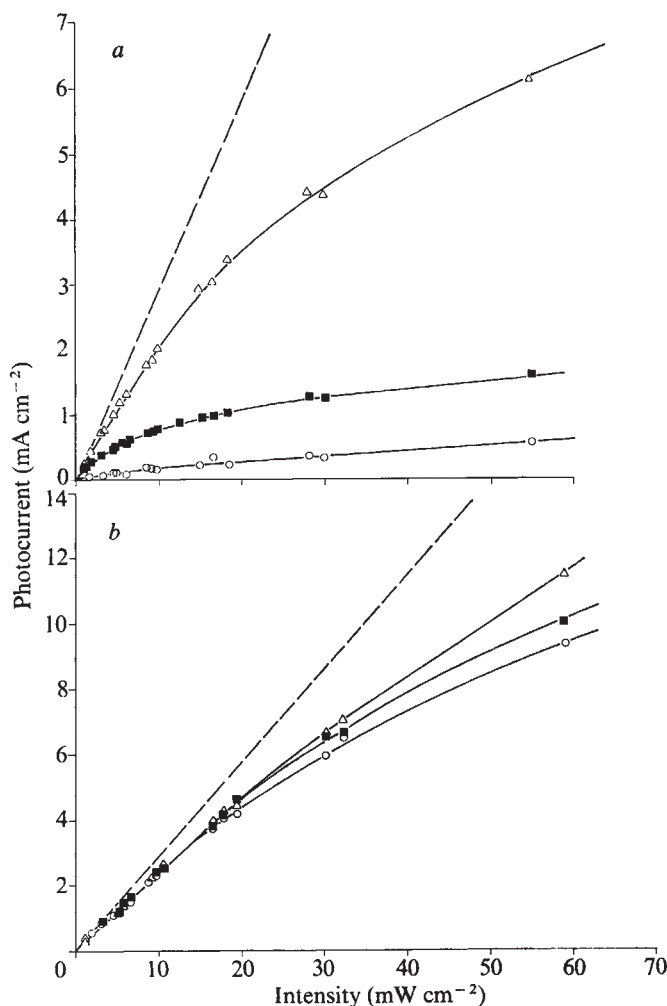


Fig. 2 The intensity dependence of the photocurrent produced on irradiation of the n-TiO_2 electrode (a), at 0 V (SCE), (b) at +1 V (SCE). ($\circ$) 0.2 N H_2SO_4 , $\kappa = 41.8 \times 10^{-3} \Omega^{-1} \text{ cm}^{-1}$; ($\blacksquare$) 0.8 N Na_2SO_4 , $\kappa = 41.3 \times 10^{-3} \Omega^{-1} \text{ cm}^{-1}$; (Δ) 0.225 N NaOH, $\kappa = 41.0 \times 10^{-3} \Omega^{-1} \text{ cm}^{-1}$.

The photoactive region for n-TiO_2 is 300–400 nm (ref. 4). A total of $\sim 2 \text{ mW cm}^{-2}$ of light in this region is available on a sunny day in our vicinity⁶. On the assumption that the quantum yield and intensity dependence are relatively independent of wavelength over this region, Fig. 2 can be used as an indication of the efficiency in the conditions specified. At 0 V (SCE) only TiO_2 electrodes in strongly basic solutions are efficient in converting light energy into electricity. Moreover the efficiency will be dependent on the nature of the electrolyte, and if OH^- is consumed in the photolysis, it will decrease with time. At an applied potential of 1 V (SCE) however, the efficiency of the TiO_2 system will be high and independent of solution pH. In addition, since the quantum yield remains relatively independent of intensity up to 12 mW cm^{-2} , some focusing of the incident radiation is allowed without a corresponding decrease in quantum efficiency.

The fact that the quantum efficiency approaches unity at low intensities for these wavelengths is significant since it suggests that further research should be directed towards broadening this wavelength range. Materials which had a photoactive region extending into the visible could utilise a larger proportion of the incident solar radiation.

Electrochemical oxidation of titanium metal to form an oxide layer⁷, and chemical vapour deposition of volatile titanium compounds to produce a polycrystalline TiO_2 layer on suitable substrates², have both been proposed as

cheaper alternatives to single crystal electrodes. The intensity dependence of the photocurrent at these electrodes can be expected to have a major role in evaluating their performance relative to the single crystal electrodes.

JOHN H. CAREY
BARRY G. OLIVER

Water Chemistry Section,
Process Research Division,
Canada Centre for Inland Waters,
Burlington, Ontario, Canada

Received November 17, 1975; accepted January 13, 1976.

- ¹ Fujishima, A., and Honda, K., *Nature*, **238**, 37–38 (1972).
- ² Hardee, K. L., and Bard, A. J., *J. electrochem. Soc.*, **122**, 739–742 (1975).
- ³ Yoneyama, H., Sakamoto, H., and Tamura, H., *Electrochim. Acta*, **20**, 341–345 (1975).
- ⁴ Wrighton, M. S., et al., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1518–1522 (1975).
- ⁵ Gerischer, H., *J. electrochem. Soc.*, **113**, 1174–1181 (1966).
- ⁶ Luckiesh, M., *Germicidal, Erythmal and Infrared Energy*, (Van Nostrand, New York, 1946).
- ⁷ Keeney, J., Weinstein, D. H., and Haas, G. M., *Nature*, **253**, 719–720 (1975).

Effect of nitrogen-containing plasmas on stability, NO formation and sooting of flames

We have been developing methods for burning leaner fuel/air mixtures at faster rates and with less pollution than is achieved in conventional systems^{1–3}. Mixtures of heat contents much below those corresponding to normal limits of flammability have been burned successfully at low temperatures using heat recirculation^{4,5}. At temperatures much above the melting point of heat exchangers large increases in burning rates for a small input of electricity were achieved when radicals from plasma jets were injected, using a magnetically rotated arc to achieve rapid mixing⁶. We now report on further effects which can be induced using even a much smaller plasma jet (of about the size of a conventional sparking plug), the addition of much smaller proportions of electrical energy (~ 4% of the chemical throughput) and the absence of any magnetic field.

In an effort to approach practical conditions more closely, the combustion chamber was a model of a gas turbine flame tube provided by Ricardo & Co. Ltd, consisting of a cylindrical outer chamber (15 × 60 cm) fitted with a Perspex end-viewing window. The fuel is injected through a 'Sonicore' nozzle into a Pyrex mixing tube, where the combustion air from a centrifugal blower flows at velocities much in excess of those used in

Fig. 1 Flame stabilised by nitrogen-containing plasma.

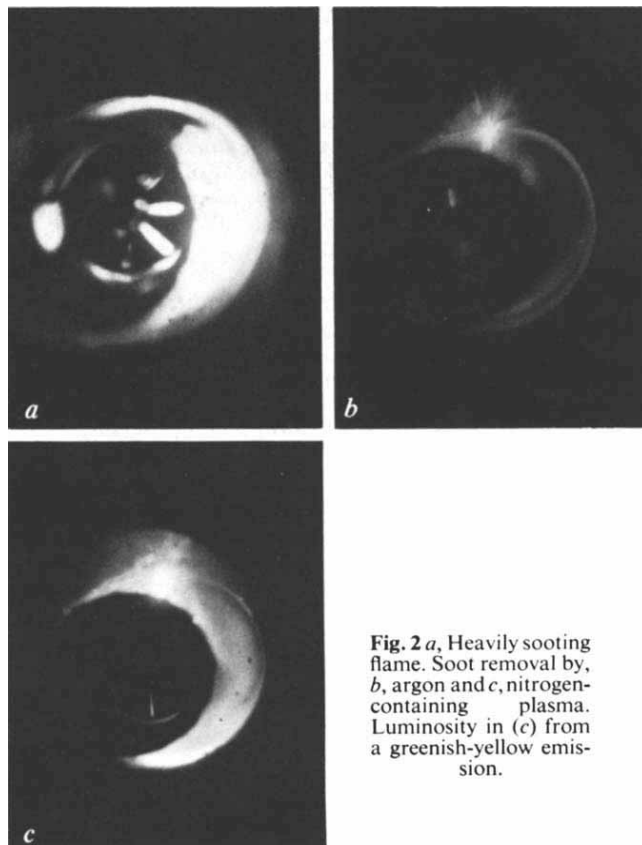
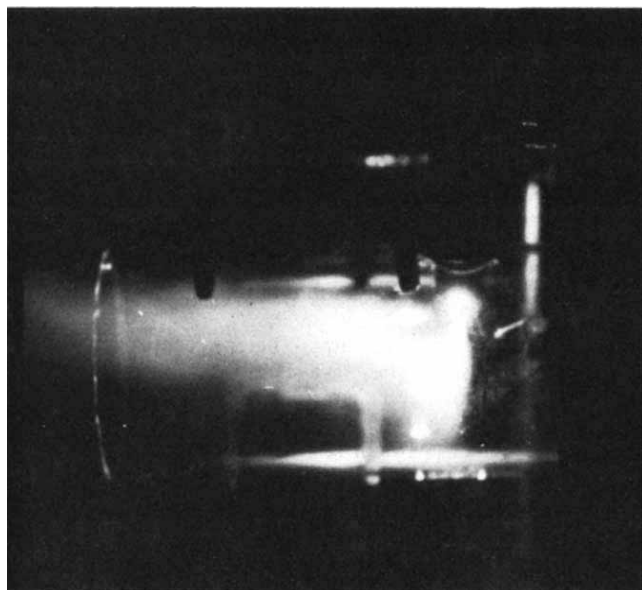


Fig. 2 a, Heavily sooting flame. Soot removal by, b, argon and c, nitrogen-containing plasma. Luminosity in (c) from a greenish-yellow emission.

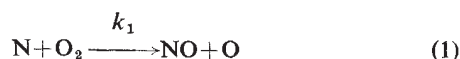
previous work. Swirl vanes upstream of the nozzle aid mixing (these are visible in Fig. 2a–c).

To allow comparison with earlier work, nitrogen-containing plasmas were used (these had been found⁶ to give marginally the best results) and were compared with 'inert' plasmas of argon alone. Once again, very large increases in stability were observed, to the point where flames could be maintained in mixtures so lean and flow velocities so high that normal flame stabilisation was impossible. Figure 1 shows such a flame anchored entirely to the nitrogen atom-containing wake of the plasma stream.

A new observation was the total disappearance of soot in the presence of the plasma, even when the latter was of argon alone and even in otherwise heavily sooting conditions. To achieve soot formation, toluene was injected with the propane fuel into the atomiser of the combustion chamber and Fig. 2a gives some impression of the luminosity of the flame which accompanied the heavy sooting. The result of injecting the argon plasma (Fig. 2b) is shown chiefly because colour photographs could not be published: the nitrogen-containing plasma (Fig. 2c) removes soot completely, probably under even more extreme conditions than does the argon plasma. There is, however, some luminosity visible in Fig. 2c: a greenish-yellow glow (also apparent under the conditions of Fig. 1) which is thought to result from some of the reactions of nitrogen atoms. This glow is seen to spiral down the length of the combustion chamber, because of the swirl vanes. Carbon luminosity disappears entirely in all cases, though black and white photographs do not show these distinctions.

Both chemical and physical mechanisms could account for soot suppression and the results, at this stage, do not allow us to attribute the effect uniquely to one or the other. Oxygen atoms (see below) and the OH produced in the next part of the radical chain could have this effect? but free electrons, which would be abundant in the plasma, have also been shown active in this way⁸.

It has been suggested that as regards flame stabilisation, at least, one of the relevant reaction steps is



There are likely to be several others, and even radiation from the plasma may exercise an effect. For example, it has been shown⁶ that argon, though much less effective than nitrogen, is far from acting as an inert additive. Because of its exceedingly high temperature, a variety of reactions can occur at its interface with the other reactants; acetylene, for example, was found⁶ when an argon plasma was injected into methane/air mixtures. In view of the effectiveness of nitrogen, however, it became desirable to consider whether the injection of nitrogen-containing plasmas would aggravate NO formation. Plasma-generated

In view of the success of these laboratory experiments, a car-battery operated plasma jet was tested in the exhaust system of a 1,200 cc saloon car and a considerable reduction of NO emission was achieved.

How flame stabilisation and NO removal can be associated becomes a little clearer if we consider the temperature dependence of the above-mentioned reactions. If one considers reaction (1) to be the main competitor for N atoms, and neglects the reverse of (1) and (2), as well as routes involving N atoms leading indirectly to nitric oxide, the following expression results

$$-d[\text{NO}]/dt = [\text{N}]\{k_2[\text{NO}] - k_1[\text{O}_2]\}$$

Therefore if $k_2[\text{NO}] > k_1[\text{O}_2]$, then nitric oxide may be destroyed at a rate proportional to $[\text{N}]$.

The temperature dependence of k_1 ($6.43 \times 10^9 T \exp(-6250/RT)$) and k_2 ($3 \times 10^{13} \exp(-334/RT)$) are shown in Fig. 3a; the low activation energy of reaction (2) makes it insensitive to temperature in the range of interest. When $k_2[\text{NO}] = k_1[\text{O}_2]$ there will be no nitric oxide reduction. By plotting $k_1[\text{O}_2]/k_2$ against temperature (Fig. 3b) it is possible to estimate a value of the lowest concentration of nitric oxide that could be established by N atom injection into nitric oxide-containing products (where the concentration was greater than the local steady state value of NO at a given temperature).

It is thus possible to postulate practical systems in which one plasma jet could fulfil both functions, one in the hot reaction zone and the other in the colder exhaust stream. This might be achieved, for example, by maintaining the discharge during parts of the combustion and exhaust cycle in a reciprocating engine, and by a suitable vortex in a continuous flow chamber.

Our current work is concerned with the analysis, by spectrographic and by other means, of the reactions occurring at the interface between plasmas of various constitutions and gases relevant in the combustion process. It seems already from these preliminary practical results, however, that it may be possible to design combustion systems in such a way that a small addition of electrical energy will facilitate the faster combustion of leaner mixtures simultaneously with the prevention of soot and nitric oxide in the products.

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J. C. HILLIARD
F. J. WEINBERG

Imperial College,
London SW7 2BZ, UK

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- ¹ Weinberg, F. J., *Phys. Technol.*, **6**, 95 (1975).
- ² Weinberg, F. J., *Phys. Bull.*, **25**, 56 (1974).
- ³ Weinberg, F. J., *15th Symposium (International) on Combustion. The Combustion Institute, Pittsburgh (1975) Plenary Lecture*.
- ⁴ Lloyd, S. A., and Weinberg, F. J., *Nature*, **251**, 47 (1974).
- ⁵ Lloyd, S. A., and Weinberg, F. J., *Nature*, **257**, 367 (1975).
- ⁶ Harrison, A. J., and Weinberg, F. J., *Proc. R. Soc.*, **A321**, 95 (1971).
- ⁷ Cotton, D. H., Friswell, N. J., and Jenkins, D. R., *Combust. Flame*, **17**, 87 (1971).
- ⁸ Bowser, R. J., and Weinberg, F. J., *Nature*, **249**, 339 (1974).
- ⁹ Zel'dovich, Y. B., *Acta phys.-chim. URSS*, **21**, 577 (1946).

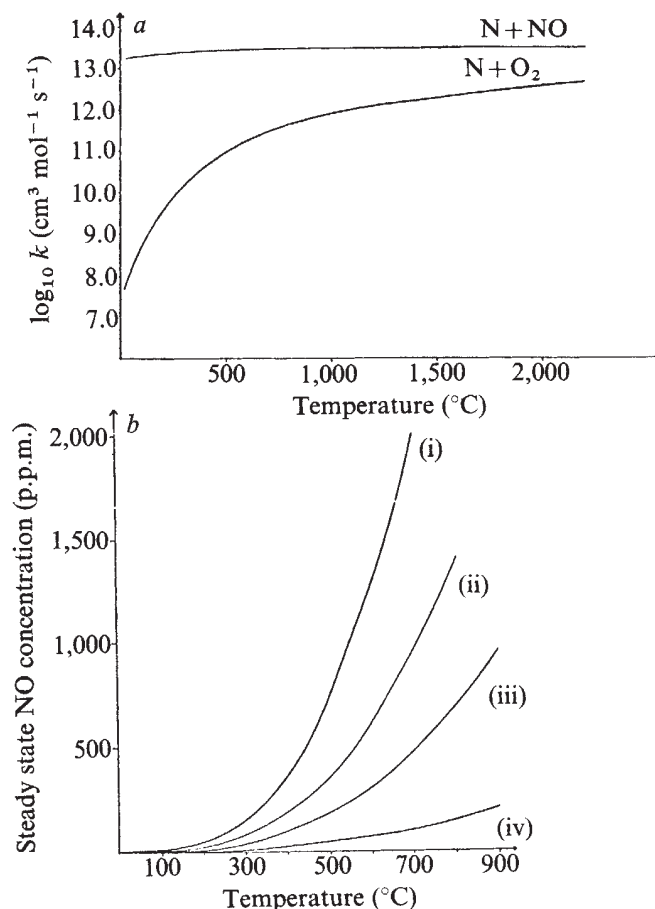
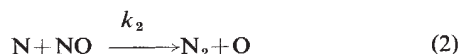


Fig. 3 Comparison of rates of reaction 1 and 2. a, Temperature dependences of rates constants; b, minimum nitric oxide concentration attainable for O_2 (i) 21% air (leanest limit), for example, an unthrottled engine at low load; (ii) 10%, unthrottled engine at high load; (iii) 5%, throttled engine, low load, high air/fuel ratio; (iv) 1%, throttled engine, idle or high load.

N atoms should, however, be able to reduce nitric oxide emissions by reversing the rate-determining Zel'dovich⁹ step



To demonstrate this reaction taking place at atmospheric pressure, N atoms from the plasma were injected into fast flowing streams of nitric oxide in argon and synthetic exhaust gas mixes. Reductions in nitric oxide from 3,000 p.p.m. to a residual 80 p.p.m. were readily obtained in flows up to 250 l min^{-1} .

The pace of life

THE specific effects of population pressure on the quality of everyday life should be of pressing social and policy concern; and although population studies have proliferated in the behavioural sciences, research has focused primarily on fertility-related behaviours^{1,2}. Moreover, the few social scientists interested in the relationship of the numbers of humans to individual human behaviour have been puzzled by a dearth of clear-cut effects³. This study reports preliminary analyses from a larger cross-cultural investigation of the quality of life. Following a suggestion from Lowin *et al.*⁴, we have systematically observed the rates of pedestrian locomotion over a constant distance in

15 cities and towns in six countries in Europe, Asia and North America. The results of these observations indicate that pace of life varies in a regular fashion with the size of the local population, regardless of the cultural setting.

Between 1972 and 1974 we had the opportunity to visit and collect observational data on the quality and pace of life from several varied locations on three continents. As part of these studies we sampled and measured the tempo at which local inhabitants simply walked the main streets of their cities and towns. Walking represents a standard human activity which previous research had suggested might be subject to the influence of local population size⁴. The locations in which data were collected were chosen to represent functionally parallel sites, 'downtown' or commercial areas like Wenceslas Square in Prague, Flatbush Avenue in Brooklyn, and Rehov Yerushalaim in Safed. In each location 50 feet (15.24 m) along the main walkway were first measured and then passers-by were timed unobtrusively from a vantage point opposite the walkway. All the data were collected on dry, sunny days of moderate temperatures (mean 24.1 °C). Subjects ($N=309$) were selected from those who walked the distance alone and unencumbered. They were selected unsystematically within these constraints and, in all cases but one, equal numbers were chosen from people walking in each direction. The data for each setting were preserved in raw form until observations on all of the 15 sites had been collected.

Figure 1 shows the mean velocities at which the inhabitants of the cities and towns traversed a constant 50-foot

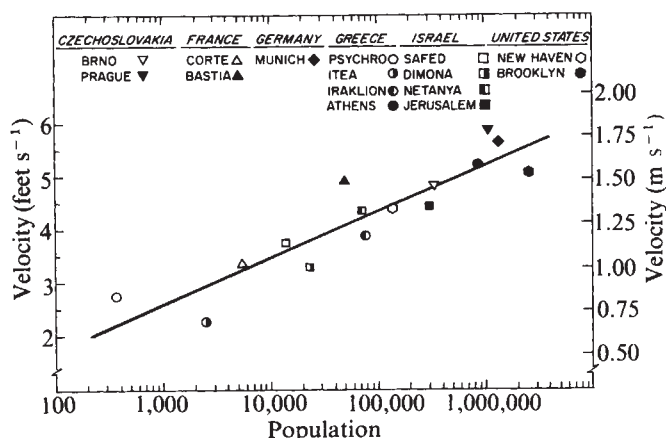


Fig. 1 Walking velocity (over 50 feet) as a function of population size. The line drawn through the data is the calculated least squares fit (linear-log regression): coefficient of determination (r^2) = 0.82. Slope, $V = 0.86 \log P + 0.05$ (V , velocity; P , population). Population values (pop.) were derived from statistical abstracts or government guides; n is the number in a sample; t is the mean time (s) (± 1 s.d.) to walk 50 feet: Czechoslovakia: Brno (pop., 341,948, $n = 20$, $t = 10.4 \pm 1.8$) and Prague (pop., 1,092,759, $n = 20$, $t = 8.5 \pm 1.3$) *Statistická ročenka CSSR* (SNTL, Prague, 1974); France: Corte (pop., 5,491, $n = 13$, $t = 15.1 \pm 1.8$) *Guide Michelin* (Pneu Michelin, Paris, 1973) and Bastia (pop., 49,375, $n = 26$, $t = 10.2 \pm 1.1$) *Annuaire Statistique de la France* (Institut National de la Statistique et des Études Économique, Paris, 1973); Germany: Munich (pop., 1,340,000, $n = 30$, $t = 8.9 \pm 1.3$) *Statistisches Jahrbuch für Die Bundesrepublik Deutschland* (W. Kohlhammer, Stuttgart, 1973); Greece: Psychro (pop., = 365, $n = 10$, $t = 18.1 \pm 4.1$) Crete by J. M. Christoforakis (S. Alexiou Sons, Iraklion, nd) and Itsea (pop., 2,500, $n = 10$, $t = 22.0 \pm 8.6$), Iraklion (pop., 78,200, $n = 20$, $t = 13.0 \pm 2.5$), and Athens (pop., 867,023, $n = 20$, $t = 9.6 \pm 1.4$) *Statistical Yearbook of Greece* (Government Printer, Athens, 1973); Israel: Safed (pop., 14,000, $n = 20$, $t = 13.5 \pm 2.5$), Dimona (pop., 23,700, $n = 20$, $t = 15.3 \pm 10.6$), Netanya (pop., 70,700, $n = 20$, $t = 11.6 \pm 2.4$), and Jerusalem (pop., 304,500, $n = 30$, $t = 11.3 \pm 1.4$) *Statistical Abstract of Israel* (Government Printer, Jerusalem, 1973); and United States: New Haven, Connecticut (pop., 138,000, $n = 20$, $t = 11.4 \pm 1.8$), and Brooklyn, New York (pop., 2,602,000, $n = 30$, $t = 9.9 \pm 1.0$) *Statistical Abstract of the United States* (US Government Printing Office, Washington, 1973).

distance. Statistical analyses showed significant variation among sample mean times to walk the distance ($F=14.69$, d.f.=14/84, $P < 0.001$). In Fig. 1, velocity is plotted against log population for the location (the total number of people living within the city limits^{5,6}). As can be seen, the relationship between walking velocity and size of the local population is suitably characterised as linear, and the coefficient of correlation between these variables ($r=0.91$) is significant beyond the 0.001 level of confidence (two-tailed test). These results suggest that a highly predictable relationship exists between the pace of life which characterises a locale and the size of its population.

Further, a comparison of cities with towns (breakpoint for a Standard Metropolitan Statistical Area⁷ > 200,000) shows that average walking time for city dwellers (mean = 9.8 s) significantly exceeded that of their town counterparts (mean = 13.4 s) ($t=7.66$, d.f.=307, $P < 0.001$). Similarly, nearly every intranational comparison indicated that city dwellers move at significantly greater speeds than their smaller town compatriots. Both these analyses further corroborate the notion that city life, at least in these Western locations, is carried on at an increased tempo⁴.

What explains the regularity with which locomotion velocity and population covary? Recent analyses of population pressures on behaviour suggest that "the number of individuals who must interact, rather than density, is the variable that produces substantial effects on human behaviour."⁶ In addition, the crowding that accompanies larger groups of humans has been defined operationally to include not only numbers of people, but also the perception of personal restriction⁸ and increased levels of social stimulation⁹. Both latter concepts covary with the size of the population. In view of this, crowding has been thought to motivate specific timing and avoidance behaviours that reduce tension and social interference. Milgram¹⁰, for example, has proposed an organising theory of psychological behaviour in cities that is based on a systems concept directly related to this view of crowding. According to his "overload" hypothesis, the volume of stimulation which characterises highly populated areas and typically overloads the processing capacity of the individual is reduced by cognitive and behavioural adaptations—usually withdrawal responses—which in turn facilitate purposeful action. These adaptive mechanisms eventually evolve into behavioural norms and, assuming this interpretation, increased walking speeds serve to minimise environmental stimulation. The data on the pace of life presented here reflect an adaptation to the continuum of population effects^{10,11}. Thus, although our samples are relatively small and the communities were not randomly selected, our data suggest that the immediate social and physical environment exert a strong control over individual behaviour. They imply that demographic circumstances affect the quality of life, psychological experience, and motor action of humans in a fairly predictable manner, even in varied cultures.

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MARC H. BORNSTEIN

Department of Psychology,
Princeton University,
Princeton, New Jersey 08540, and
Max-Planck-Institut für Psychiatrie

HELEN G. BORNSTEIN

Max-Planck-Institut für Psychiatrie,
8-Munich-40, Kraepelinstrasse 10,
West Germany

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¹ Bartz, W. R., *Am. Psychol.*, **25**, 500 (1970).

² Fawcett, J. T. (ed.), *Psychological Perspectives on Population* (Basic Books, New York, 1973).

- ³ Lawrence, J. E. S., *Psychol. Bull.*, **81**, 712 (1974).
⁴ Lowin, A., Hottes, H. A., Sandler, B. E., and Bornstein, M. H., *J. soc. Psychol.*, **83**, 247 (1971).
⁵ Wirth, L. J., *Am. J. Soc.*, **44**, 1 (1938).
⁶ Freedman, J. L., in *Psychological Perspectives on Population* (edit. by Fawcett, J. T.), 234 (Basic Books, New York, 1973).
⁷ Carnahan, D., Grove, W., and Galle, O. R., *Soc. Forces*, **53**, 63 (1974).
⁸ Stokols, D., *Psychol. Rev.*, **79**, 275 (1972).
⁹ Desor, J. A., *J. Personality soc. Psychol.*, **21**, 79 (1972).
¹⁰ Milgram, S., *Science*, **167**, 1461 (1970).
¹¹ Zajonc, R. B., *Science*, **149**, 269 (1965).

Mortality and the 1974 fuel crisis

SOME comment is needed on the report by Brown *et al.*¹ in which the authors claim that the low mortality they noted in San Francisco and Alameda Counties in the first quarter of 1974 might have arisen from the reduction of gasoline sales during the fuel crisis. This is an interesting proposition, but the further details that the authors are examining on the more usual determinants of seasonal and secular variations in mortality (weather conditions and the prevalence of respiratory infections), and on urban/rural comparisons will be required before any judgment can be made on possible cause-and-effect relationships. In the meantime we make a few comments on their work, and present some related data from Britain.

Brown *et al.* have provided very little information on which to assess the significance of the variations in quarterly death rates. The actual numbers of deaths involved have not been quoted (although these can be estimated from a knowledge of the total populations), and beyond stating that the first-quarter rates for the years 1970–73 were “rather homogeneous”, there is little indication of the extent of year-to-year fluctuations.

The comment that the greatest change occurred in the under-45 age group, and the least among those aged over 65 is surprising, in view of the low death rates in the former group. Normally, mortality in the over-65 group dominates the total death rates, and it is this group that shows the greatest changes in mortality in epidemics of influenza or in extreme weather conditions. Presumably age-specific death rates had been calculated, but these were not shown. Although it is unlikely that there would have been any change in age distribution within either area during the five years concerned sufficient to affect comparisons of total death rates from one year to another, the contrast in death rates between San Francisco and Alameda Counties suggests a major difference in age distribution between these two areas.

In view of their suggestion that the effect on mortality is related to the concentrations of pollutants from motor vehicles, it is surprising that results of measurements of, for example, carbon monoxide, have not been quoted. Apart from variations in the emission of pollutants from one period to another, the prevailing weather conditions are important in determining the actual concentrations found in streets.

Table 1 Annual death rates in Greater London for 1971–75

Year	Annual death rates* per thousand for the quarters ending			
	March	June	Sept.	Dec.
1971	12.8	11.2	10.0	11.7
1972	13.5	11.1	10.1	12.4
1973	13.3	11.0	9.9	12.0
1974	13.3	11.1	10.3	12.0
1975	13	12	10	—

*Death rates as published by the Office of Population Censuses and Surveys², subject to modification in relation to revised estimates of population, and to final adjustments of deaths in relation to exact date and place of occurrence. The numbers of deaths per quarter range from 18,000 to 25,000.

Table 2 Annual death rates in Greater London for 1955–57

Year	Annual death rates* per thousand for the quarters ending			
	March	June	Sept.	Dec.
1955	14.3	9.7	8.2	10.4
1956	14.8	9.7	8.4	10.6
1957	11.3	9.7	8.7	13.2

*Death rates as published in the Registrar General's Quarterly Returns². The numbers of deaths per quarter range from 17,000 to 30,000. Greater London was not identified separately in the records in other years around this period, and the definition of the area differs slightly from that in Table 1.

Earlier work by Hexter and Goldsmith³, based on daily mortality records in Los Angeles County, showed that a model including regression on the logarithm of the daily mean concentration of carbon monoxide was capable of accounting for a marginally greater proportion of the variance in deaths than one involving trend, cyclic variations and temperature, without added pollution variables. The interpretation of such findings is, however, difficult since some of the variables involved are correlated with one another, and in these circumstances ordinary least squares estimates of the regression coefficients are unsatisfactory³.

Relationships between daily mortality and concentrations of pollutants from heating sources (smoke and sulphur dioxide) have been studied in London^{4–6}, but the possibility of associations with pollutants from traffic have not so far been considered. Inspection of the records of death rates for each quarter of the year as published for Greater London⁷ for the past five years (Table 1) does not reveal any reduction in mortality in the first quarter of 1974 when, as in California, there was a marked reduction in the use of cars as a result of the fuel crisis (and a more important feature in London, affecting the emission of pollution both from heating and from vehicles was the limitation in the use of fuels for heating and power, with the imposition of a three-day working week).

It is of interest also to consider an earlier period, during the Suez crisis in the early months of 1957, when as a result of petrol rationing there was a major reduction in the use of cars, and substantial changes in carbon monoxide concentrations were noted in central London⁸. The death rates for Greater London on a quarterly basis during the three years 1955–57 (ref. 9) are shown in Table 2.

The relatively low mortality rate in the first quarter of 1957, and the high mortality rate in the last quarter were features common to the records for the whole of England and Wales. The rise at the end of the year was related to the Asian influenza epidemic, and the low death rate in the first quarter was attributed to the mild weather¹⁰. There have been other March quarters in recent decades with low mortality (for example 1948, 1935) and some with high mortality, related to cold weather (as in 1963 (see ref. 11)) or to influenza epidemics. Further evidence of the important effects of temperature on deaths from heart disease has been reported by Bull and Morton¹². Much caution is therefore required before ascribing changes in mortality to other environmental factors, and it is necessary to examine a long series of data with influenza epidemics, periods of extreme weather conditions, or episodes of unusually high or low pollution in a variety of combinations with one another before the relative importance of each of these factors can be judged satisfactorily.

In addition to the published quarterly and weekly returns of deaths, special daily tabulations have been prepared for London for a number of years, and more recently these have been extended to other urban areas and to England

and Wales as a whole. A detailed analysis of these is already in progress, and some of the findings on the effects of several environmental factors will be published shortly. Meanwhile we await with great interest the further results and analyses promised by the Berkeley workers.

R. E. WALLER
P. J. LAWTHOR

MRC Air Pollution Unit,
St Bartholomew's Hospital Medical College,
London EC1M 6BQ, UK

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- ¹ Brown, S. M., Marmot, M. G., Sacks, S. T., and Kwok, L. W., *Nature*, 257, 306-307 (1975).
- ² Hexter, A. C., and Goldsmith, J. R., *Science*, 172, 265-267, (1971).
- ³ McDonald, G. C., and Schwing, R. C., *Technometrics*, 15, 463-481 (1973).
- ⁴ Gore, A. T., and Shaddick, C. W., *Br. J. prev. soc. Med.*, 12, 104-113 (1958).
- ⁵ Martin, A. E., *Proc. R. Soc. Med.*, 57, 969-975 (1964).
- ⁶ Waller, R. E., Lawther, P. J., and Martin, A. E., *Proc. Clean Air Conf., Eastbourne*, 71-79. (National Society for Clean Air, London, 1969).
- ⁷ Office of Population Censuses and Surveys. *Population trends*, No. 2. (HMSO, London, 1975).
- ⁸ Lawther, P. J., Commins, B. T., and Henderson, M., *Ann. occup. Hyg.*, 5, 241-246 (1962).
- ⁹ Registrar General. *Quarterly return for England and Wales*, Nos. 425-436 (HMSO, London 1955-58).
- ¹⁰ Registrar General. *Statistical Review of England and Wales for the year 1957. Part III, Commentary*, 59-62. (HMSO, London, 1959).
- ¹¹ Registrar General. *Statistical Review of England and Wales for the year 1963. Part III, Commentary*, 162-168. (HMSO, London, 1966).
- ¹² Bull, G. M., and Morton, J., *Age Ageing*, 4, 19-31 (1975).

THE paper by Waller and Lawther¹ commenting on our report of a decrease in mortality during the 1974 fuel crisis² raises some interesting and important points. We chose two separate countries to examine the possible effects on mortality of the fuel crisis for the purposes of testing consistency. Although both Alameda and San Francisco Counties are in the San Francisco Bay Area, they vary somewhat in age and racial composition, in density of population, in occupational structure, and in weather conditions. Yet both counties showed a drop in mortality during the first quarter of 1974.

Why no such mortality decrease was seen in London

on the basis of variation in such factors, but we have not yet investigated the possible interaction of all these factors.

A further possibility is that the decrease in mortality is part of a secular trend. We should be in a better position to answer this question when later data become available.

Waller and Lawther suggest that measurements of specific pollutants ought to have been presented. For several reasons we decided that our investigation should not hinge on variations in observed pollutant levels. First, we have become aware of significant technical problems in the actual measurement and recording of pollutant levels. Further, in San Francisco and Alameda Counties, there are only five air-sampling stations for a total population of one-and-three-quarter million people. Thus, reported pollutant levels are at best a very indirect guide to human exposure. Also, one must distinguish between the exposure of the general population to ambient levels of pollutants and the localised over-exposure of people in and around motor vehicles. We do, however, intend to publish ambient pollutant levels in the larger paper to follow. In response to Waller and Lawther's other questions: the number and rates of deaths per quarter are given in Table 1.

It should be stressed that the data from the two counties were collected differently. The Alameda County deaths are reported by place of occurrence, and for that county the figures in Table 1 should be accurate. The San Francisco deaths are reported by place of residence. As the San Francisco figures are from early reports, they may not include some deaths among San Francisco residents who died out of the county, and death reports received by the Registry after March 31, 1974. When these latter deaths are included, the 1974 mortality figure for San Francisco (but not for Alameda) may be higher than the one quoted.

Waller and Lawther are correct in noting the great absolute difference in the death rates between these two counties. This difference cannot be wholly explained by the older age distribution in San Francisco, but is presumably the result of other ethnic and environmental factors. We

Table 1 First quarter deaths and death rates per 100,000 population. All causes excluding motor vehicle accidents

	1970	1971	1972	1973	1974
Alameda County*					
Number of deaths	2,122	2,184	2,102	2,256	2,019
Quarterly mortality rate	225.3	230.0	219.3	234.2	209.7
San Francisco County					
Number of deaths	2,682	2,610	2,631	2,608	2,221
Quarterly mortality rate	374.8	371.3	381.6	382.7	327.0

* Excludes the cities of Berkeley and Albany, which have separate death registries.

during the first quarter of 1974 is not clear. One may speculate that the three-day working week and the consequent social disruption, the apparent decrease in home heating, and many other factors might have affected overall mortality in a way that would 'mask' any effect on mortality of the decrease in gasoline consumption.

The other possibility for the discrepancy between our findings and the London findings is that raised by Waller and Lawther, that is, factors other than the change in gasoline consumption may have been responsible for the decrease in mortality (in the San Francisco Bay Area). We have examined separately the variation in rainfall, temperature and air stability that occurred in the first quarters of 1970-74 and in each case 1974 was fairly typical. Similarly, the change in the number of deaths from influenza and pneumonia over the 1970-74 period does not seem to vary with the mortality rates of the counties in question. We have not been able to explain the decreased mortality in 1974

have taken account of the change from year to year in the population size of each county, but not of any change in internal composition, as such figures were not available to us. Our assertion that the greatest proportionate change occurred for those under the age of 45 is based on the percentage change using the average death rates for the comparison years as baseline. Of course, the absolute rates are much higher in those over the age of 65 but the greatest proportionate change was in the younger age groups. This preliminary analysis of the effect within age strata was not simultaneously tested by race and sex; consequently, no clear-cut conclusions can be drawn until this is accomplished.

We are heartened that other investigators have begun to look at populations and areas very different from our own to corroborate or refute our findings. We sincerely hope that these efforts will continue. We would hope that analysis by cause of death, and of the other factors which may mag-

nify or diminish observable changes in mortality during the fuel crisis period will also continue.

M. G. MARMOT
S. M. BROWN
S. T. SACKS
L. W. KWOK

School of Public Health,
University of California, Berkeley,
California 94720

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¹ Waller, R. E., Lawther, P. J., *Nature*, 259, 559–560 (1976).

² Brown, S. M., Marmot, M. G., Sacks, S. T., Kwok, L. W., *Nature*, 257, 306–307 (1975).

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RICHARD R. TENAZA

Department of Biological Sciences,
University of the Pacific,
Stockton, California 95211

Received October 6; accepted December 10, 1975.

¹ Thorpe, W. H., *Proc. zool. Soc. Lond.*, 132, 441–455 (1959).

² Bertram, B., *Anim. Behav. Monogr.*, 3, 81–192 (1970).

³ Tenaza, R. R., *Folia Primatol.*, 24, 60–80 (1975).

⁴ Tenaza, R. R., *Z. Tierpsychol.*, 40, 37–52 (1976).

Wild mynahs mimic wild primates

THE remarkable accuracy with which captive hill mynahs (*Gracula religiosa*) mimic human speech and other sounds is well known¹. How this ability functions in nature was entirely speculative until Bertram² conducted an extensive field study of hill mynahs in India. Bertram² found that hill mynahs in India imitate the calls of other hill mynahs, but never heard a wild mynah imitate another species.

A different situation prevails on Siberut Island, off the west coast of Sumatra, Indonesia. During a 14-week study (1 July–7 October, 1972) of primates in rain forest near the Sirimuri River in southern Siberut³, I noted several instances of wild *G. religiosa* imitating a particular loud alarm call of Kloss's gibbon (*Hylobates klossii*) and loud spacing call of male pig-tailed langurs (*Simias concolor*).

I saw hill mynahs daily in the study area, usually in groups of 2–10 individuals. The gibbon call that they imitated is a tonal trill at a frequency of ~ 1 kHz and an average duration of 1 s (21 sonagrams). The langur call that they mimic is a panting-like sound, produced by 3–5, or more, exhalation–inhalation cycles, at a frequency of ~ 500 Hz (1 sonagram). Mynah imitations of these calls sound much like the originals but are distinguishable by ear as being mynah calls rather than primate calls just as readily as mynah renditions of human speech can be recognised by ear.

Mynahs imitated calls immediately after the primates produced them. From 16–30 September, 1972, I noted the frequency with which male pig-tailed langur calls were followed by mynah imitations of the calls. Out of 12 instances of calling by one or more male pig-tailed langurs, 10 were followed within seconds by imitations of the call by one or more mynahs. Comparable data were not obtained for mimicking of gibbon alarm trills because I heard none during this period.

Neither gibbon alarm trills nor the male pig-tailed langur call was a common component of the forest background noise during this study. Only 12 bouts of gibbon alarm trills, distributed over 8 days, were heard during the 99 days of study. Pig-tailed langur calls were never heard during the first 11 weeks of the study, but occurred on two-thirds of the days during the final three weeks of study. R. L. Tilson (in preparation) has confirmed that calling by pig-tailed langurs occurs sporadically.

Three kinds of loud primate calls are, at particular times of day, regular components of background noise in the study area. These are the songs of male and female⁴ Kloss's gibbons and spacing calls of male Mentawai langurs (*Presbytis potenziani*). None of these calls was imitated by mynahs, in spite of the fact that the Mentawai langur call is rather similar to the pig-tailed langur call which is mimicked. This suggests that perhaps only infrequently occurring vocal signals of other species are mimicked by mynahs. I did not determine whether mynahs imitate vocalisations of other species of birds in the study area.

These findings suggest that such mimicking behaviour is not, as previously thought², just an artefact of confinement.

Wild birds detect quinine on artificial Batesian models

EVIDENCE I acquired during an experiment on the evolution of Batesian mimicry indicated that under some circumstances birds can detect quinine dihydrochloride on flour–lard pastry baits. The results of some previous mimicry experiments^{1–5}, in which pastry baits were presented to wild birds, may need to be re-evaluated because of their tacit assumption that birds can only detect by taste the quinine used to make the models unpalatable. Quinine salts used were the hydrochloride^{2,3} or dihydrochloride^{1,5}. Pilecki and O'Donald's "solution of quinine"⁴ was presumably quinine hydrochloride, which they used previously. Quinine monohydrochloride has only 1:16 solubility⁶, so Morrell and Turner² probably used the more soluble dihydrochloride to make their 75% solution. Birds in an experiment of Ikin and Turner¹ took nearly as many perfect mimics of two different models as palatable controls, though very few models (70% quinine) were taken. These authors suggested that birds in previous experiments^{2,5} may have distinguished "models from 'perfect' mimics, possibly because the quinine causes a slight yellowing of the pastry". So far as I can determine, however, a specific control for detection of quinine has never been conducted.

The intent of my original experiment was to test the hypothesis that the models evolve more slowly than the mimics due to stabilising selection⁷; that is, deviant individual models are subject to predation because birds fail to recognise them as unpalatable. Until my suspicions about detection of quinine by birds were aroused, I used the following procedure in the study. Birds were trained to avoid unpalatable yellow models and palatable (Batesian) yellow mimics, and to choose palatable pink controls. All baits were painted with black patterns. The position of a single black stripe distinguished models from mimics, while controls were painted with four other patterns. I planned next to test avoidance of models with altered black patterns. During training, two scrub jays (*Aphelocoma coerulescens*) avoided yellow model baits treated with a 5% quinine solution (Q-yellow) and yellow mimics (non-Q-yellow, dipped in distilled water). They chose controls (non-Q-pink, distilled water) significantly more than models and mimics combined ($\chi^2 = 127.12$, $P < 0.001$, see Table 1b). Other birds feeding on the baits—6–20 Golden-crowned sparrows (*Zonotrichia atricapilla*), two to six brown towhees (*Pipilo fuscus*), one to two rufous-sided towhees (*P. erythrophthalmus*) and one to two California thrashers (*Toxostoma redivivum*)—did not discriminate among model, mimic and control. I increased the unpalatability of the model, using 60% quinine dihydrochloride for the rest of the experiment. The birds other than jays then chose non-Q-yellow mimics significantly more than Q-yellow models ($\chi^2 = 29.35$, $P < 0.001$, Table 1c). The jays continued to avoid both Q and non-Q-yellow baits significantly.

The first indication that some birds detected quinine visually or olfactorily was that only 12 Q-yellow baits were taken during the 4 d when 60% quinine was used initially, although at least 17 birds in addition to jays were seen feeding on the first day alone (Table 1c). I was certain that the birds did not taste baits without attacking them. I used field glasses to watch

Table 1 Experimental details and results

Date (1973)	Model	Mimic	Control	No. presented	No. taken, all birds	No. taken, scrub jays only
(a) January 19, 20, 22–24	5% Q-yellow			250	109	39
			non-Q-pink	250	202	127
(b) January 27, 28, 31	5% Q-yellow			300	66	
		non-Q-yellow		300	76	2 yellow*
			non-Q-pink	300	218	133
(c) February 15–18	Q-yellow			200	12	0
		non-Q-yellow		200	57	0
			non-Q-pink	200	160	102
(d) March 15	Q-pink			50	0	0
		non-Q-pink		50	43	33 pink*
			non-Q-green†	50	21	16

Tests were run at Stanford between 0800 and 1000 each fair day between January 19 and March 28, 1973, and sessions were continued for 20 min or until about 10 of the most heavily predated class of baits remained. Baits were presented in random order, 10 cm apart in a rectangular or square array on a secluded lawn, and were not replaced when taken. Feeding was continuously observed during sessions; the number of each colour taken by each species was recorded. A test for colour preference had been conducted for 5 d before January 19. Given 50 unpatterned non-Q-pink and 50 unpatterned non-Q-yellow baits per session, the birds ate more of the latter, although the difference was not significant. Models were then designated yellow. Treatment of models was with 60% quinine, except during (a) and (b).

*Q and non-Q indistinguishable through field glasses, but jays must have taken only non-Q-pink during (d), since no models were taken by any birds.

†Green baits were not cryptic on the dormant grass.

Table 2 Quinine detection test

Date (1973)	Unpatterned baits	No. presented	No. taken, all birds	No. taken, scrub jays only
March 22	Q-pink	50	1	
	non-Q-pink	50	39	22*
March 28	Q-yellow	50	3	2†
	non-Q-yellow	50	20	0

Since mimics became the preferred prey in the previous test, non-Q controls made of different coloured pastry were no longer necessary. Differences shown are all significant at the 0.001 level except predation on yellow by jays. χ^2 values for predation on Q-pink against non-Q-pink: jays, 18.18; other birds, 14.22. For predation on Q-yellow against non-Q-yellow by birds other than jays, $\chi^2 = 17.19$.

*Indistinguishable, but at most one was Q-pink.

†Determined by my retrieving and tasting baits after jays dropped them.

all feeding, and the birds never brought the tips of their beaks close to one bait and subsequently chose another, nor were there beak marks on baits that were not taken.

When it was obvious that a specific test for perception of quinine was necessary, the birds were trained for 3 d on unpatterned, non-Q-pink and non-Q-green baits to accustom them to new green controls. I then tested avoidance of a new model—Q-pink baits painted with the same black pattern as the previous yellow model. All birds avoided them (Table 1d). Although they had never before seen a quinine-treated pink bait, these birds could distinguish between Q-pink and non-Q-baits. To determine whether this discrimination was made by learning the patterns that had been painted on the baits or by perceiving the quinine, unpatterned pink baits—50 Q and 50 non-Q—were presented in random order, 10 cm apart in a square array. Position within the square identified uneaten baits as to treatment; baits not taken remained undisturbed by the birds. Table 2 shows that discrimination was based solely on the presence of quinine. The test was repeated using unpatterned Q-yellow and non-Q-yellow baits. Although the jays had so far taken a mean of 36.5 baits per session, they took only two baits, subsequently identified as quinine-treated. The other birds avoided the Q-yellow baits ($P < 0.001$).

Clearly these birds detected 60% quinine-treated baits without tasting. Since jays distinguished Q from non-Q baits when the pastry was pink and were apparently unable to do so with yellow pastry, their discrimination was probably made on a visual, rather than an olfactory basis. That jays took only non-Q-pink controls during the aborted experiment (Table 1c) was a further indication that they could not distinguish between Q-yellow and non-Q-yellow baits, although the other

birds could do so with 60% quinine. Future experiments on mimicry using pastry baits must include a control to determine that the predators cannot perceive the unpalatable substance itself if baits are merely dipped into a solution. The unpalatable compound may have to be injected into baits, as Alcock⁸ did with quinine-treated mealworms, or otherwise hidden within the pastry. This is effective if the birds do not swallow food whole, but break it up and thus taste the quinine. For birds to learn to avoid a model, it must have a punishing aspect, either an unpleasant taste or a distinct taste combined with emesis, such as quinine sulphate⁹ provides.

The birds in my investigation made a very fine discrimination; I could not see the quinine on the baits. Certainly birds can and do make choices based on very small differences between food items; for example, cliff swallows have been known to select the non-stinging drones among flying bees¹⁰. It has, however, been pointed out¹¹ that predators do not need to avoid models and mimics completely for mimicry to evolve—only that better mimics must be avoided more frequently than less perfect mimics.

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ELINOR CRUZE TERHUNE*

Department of Biological Sciences,
Stanford University,
Stanford, California 94305

Received August 18; accepted December 5, 1975.

*Present address: Department of Entomology, Comstock Hall, Cornell University, Ithaca, New York 14853.

¹ Ikin, M., and Turner, J. R. G., *Nature*, **239**, 525–527 (1972).

- 2 Morrell, G. M., and Turner, J. R. G., *Behaviour*, 36, 116-130 (1970).
 3 O'Donald, P., and Pilecki, C., *Evolution*, 24, 395-401 (1970).
 4 Pilecki, C., and O'Donald, P., *Evolution*, 25, 365-370 (1971).
 5 Lea, R. G., and Turner, J. R. G., *Behaviour*, 42, 131-151 (1972).
 6 Sollman, T., *A Manual of Pharmacology and its Applications to Therapeutics and Toxicology*, eighth ed. (Saunders, Philadelphia, London, 1957).
 7 Nur, U., *Am. Nat.*, 104, 477-486 (1970).
 8 Alcock, J., *Behaviour*, 40, 1-9 (1971).
 9 Alcock, J., *Anim. Behav.*, 18, 592-599 (1970).
 10 Grant, C., *Condor*, 47, 261-263 (1945).
 11 Rothschild, M., and Kellett, D. N., *J. Entomol.*, A46, 103-110 (1972).

Aggregation response of nymphs to pheromone(s) produced by males of the tick *Amblyomma hebraeum* (Koch)

No aggregation response by tick nymphs to a pheromone(s) produced by males had been described, although such a response had been demonstrated in adults¹⁻⁸. We have now shown that males of the hard tick *Amblyomma hebraeum* (Koch) produce a material(s) which induces aggregation of nymphs suggesting an assembly pheromone(s) (Fig. 1). The presence of this pheromone is demonstrated by biological observations and by experimentation with a choice chamber⁹.

Males of *A. hebraeum* which readily attach to the host, were placed on the ears and on shaved areas of the back of rabbits (*Lepus europaeus* Pallas) and on the ears and scrotum of Guernsey calves. Hexane extracts were made by washing males of *A. hebraeum*, which had previously fed for 8 d on a calf. The extracts were concentrated to 1 ml (100 males) by vacuum distillation and were applied, drop by drop, discrete areas (a circle about 3 cm in diameter) on the ears and backs of rabbits, and on the ears

Fig. 1 Assembly of *Amblyomma hebraeum* nymphs (a) to male ticks which had attached to the host 8 d previously (b), to hexane extract of fed males. The method of extraction was described previously⁹.

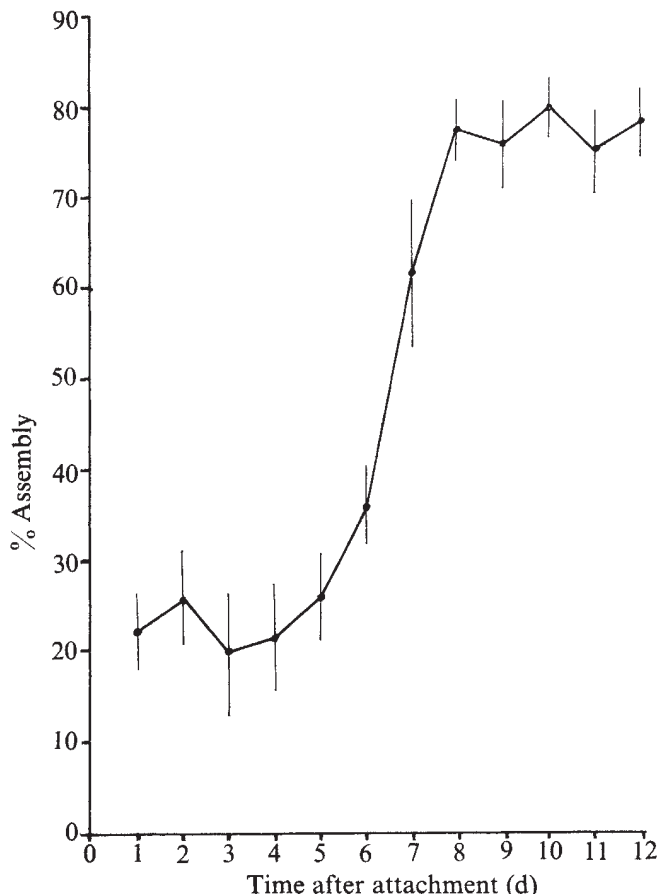
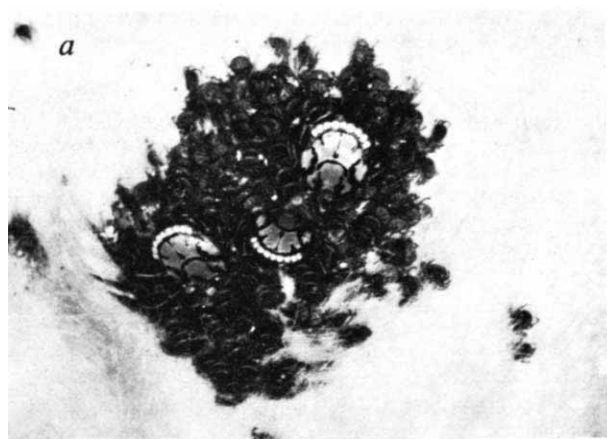


Fig. 2 Percentage assembly of *A. hebraeum* nymphs to males after various periods of feeding on scrotum of calves. Vertical line represents s.e.m.

and scrotum of calves. Test areas were enclosed with large cloth sleeves and a relatively large number of unfed nymphs was deposited in the sleeves. After 24 h the sleeves were removed and the number and area of attachment of the nymphs were recorded (Table 1). No nymphs released on a host aggregated around females which had attached to a host 5 d previously or to nymphs which had attached 4 d previously.

As a control, nymphs were exposed in a similar manner to hosts which were not infested with males or were not treated with hexane extracts of males. On control hosts, nymphs attached readily, but were evenly distributed through the area covered by the cloth sleeves and no clusters of attaching nymphs were formed.

As free blood or damaged tissues around the site of attachment might have resulted in attraction of nymphs, wounds were created on the ears of rabbits using a sharp needle. Nymphs in cloth sleeves exposed to ears treated in this way showed no clustering of attaching nymphs to either free blood or damaged areas.

The time required by nymphs for attachment to the hosts on which males were attached, or to areas of the hosts which had been treated with hexane extracts of fed males, was significantly ($P < 0.005$) shorter than in untreated control animals. The percentage of males emerging from nymphs which developed on hosts which had males attached, was compared with the percentage of males emerging from nymphs which developed on control animals. From all the tests examined $46.4 \pm 8.21\%$ were males from nymphs which attached in clusters in association with a male and $49.4 \pm 6.85\%$ were males from those that emerged from nymphs which had not aggregated around a male on the host. This small difference was subjected to statistical analysis using the t test and found to be not significant.

Table 1 Rate of assembly of *A. hebraeum* nymphs around *A. hebraeum* males

Area of host	% Assembly of nymphs (mean±s.e.) around males which had attached to the hosts 8 d previously	% Assembly of nymphs (mean±s.e.) attached to area where hexane extract of males was previously applied	% Assembly of nymphs (mean±s.e.) attached to area where pure hexane was previously applied
Rabbit ear	82.1±4.02	86.5±3.72	7.2±1.78
Rabbit back	93.3±1.71	92.1±2.01	9.2±3.12
Calf ear	93.6±1.60	84.4±3.86	6.5±2.08
Calf scrotum	79.5±3.34	90.9±4.72	2.9±1.45

This clearly indicates that all nymphs are attracted to a mature male on the host, irrespective of whether they are destined to develop as males or females.

The unfed males on the host did not attract nymphs, so further examination was necessary to determine the duration of the feeding period before males would attract nymphs (Fig. 2). Larvae did not respond to the hexane extracts of adult males to which nymphs clearly responded.

In each of the tests in the choice-chamber 100 unfed nymphs were placed in the lower arm of a Y-shaped glass tube, the ends of which were enclosed with fine nylon mesh. By connecting a vacuum pump to the lower arm of the Y-tube an airflow was produced. Tick nymphs were introduced to the lower arm while test materials were placed on the anterior arms of the choice chamber. In each test two materials were compared and three counts of the distribution of the nymphs were made at 10-min intervals. After three counts all nymphs in the system were collected and reintroduced to the lower arm of the Y-tube. Each test was replicated six times. The various materials that were checked in the choice chamber were: fed males, partly engorged females, partly engorged nymphs, unfed males, unfed females, unfed nymphs, hexane extract of males and pure hexane.

The results of a series of tests showed significant ($P < 0.002$; t test for paired observations) attraction of nymphs only in the tests in which fed males or hexane extract of fed males were present.

We have shown that males which fed for 8 d, or the hexane extract from such males significantly attracted nymphs, whereas males which had not been fed, or partially engorged females as well as partly engorged nymphs presented no attraction to nymphs.

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Y. RECHAV
G. B. WHITEHEAD
M. M. KNIGHT

Tick Research Unit,
Department of Zoology and Entomology,
Rhodes University,
Grahamstown, South Africa

Received October 20; accepted December 19, 1975.

- ¹ Gladney, W. J., *Nature*, **232**, 401 (1971).
- ² Berger, R. S., Dukes, J. C., and Chow, Y. S., *J. med. Ent.*, **8**, 84 (1971).
- ³ Berger, R. S., *Science*, **177**, 704 (1972).
- ⁴ Leahy, M. C., Vandehey, R., and Galun, R., *Nature*, **246**, 515 (1973).
- ⁵ Gladney, W. J., Grabbe, R. R., Ernst, S. E., and Oehler, D. D., *J. med. Ent.*, **11**, 303 (1974).
- ⁶ Sonenshine, D. E., Silverstein, R. M., Layton, E. C., and Homsher, P. J., *J. med. Ent.*, **11**, 307 (1974).
- ⁷ Gladney, W. J., Ernst, S. E., and Grabbe, R. R., *Ann. ent. Soc. Am.*, **67**, 750 (1974).
- ⁸ Chow, Y. S., Wang, C. B., and Lin, L. C., *Ann. ent. Soc. Am.*, **68**, 485 (1975).
- ⁹ Samish, M., *Entomophaga*, **18**, 169 (1973).

Position-dependent development of tobacco meristems

THE apical meristems of angiosperms exhibit indeterminate growth during vegetative development and determinate growth in the production of thorns and flowers¹. The mechanism(s) controlling the conversion from indeterminate

to determinate growth is poorly understood, although photoperiod and temperature have been shown in many plants to be important environmental signals for flower induction². In 'day-neutral' plants, which are not strictly regulated by photoperiod, the conversion of their meristems to the determinate flowering condition seems to be endogenously regulated. We have examined this conversion in *Nicotiana tabacum* cv. Wisconsin 38, a day-neutral plant. Our data show that a uniform number of nodes is produced before conversion, and this pattern of meristem development is regulated by information supplied to the meristem by the plant.

Wisconsin 38 tobacco exhibits limited growth because its terminal meristem differentiates into a flower. It has strong apical dominance and its vegetative axillary buds are all arrested after the initiation of eight or nine leaf primordia. In certain environmental conditions the number of nodes produced by the terminal meristem is very uniform³, and we have observed this pattern of growth in numerous plants at various times of the year. In one set of thirty plants, an average of 35.4 nodes was produced (s.d.=1.0).

We examined the conversion from indeterminate to determinate growth by taking the number of nodes produced by axillary buds (a single bud in the axil of each leaf) as a function of their position on the main axis. In our first experiments, plants at anthesis (first flower open) were decapitated at various internodes and the uppermost axillary bud was allowed to develop. These buds replaced approximately the number of nodes removed, with the higher buds producing fewer nodes than the lower buds (Table 1). This differential growth response might be related to the developmental state of the plant, and so we decapitated some plants at 3 stages before anthesis; when the inflorescence was visible, when the terminal meristem had just become reproductive, and when it was still vegetative (Table 1). In all cases the higher buds produced fewer nodes, suggesting that

Table 1 Nodes produced by terminal and axillary buds

State of development of decapitated plant*	Node number of bud that developed after decapitation	No. of nodes produced by bud in column two (± s.d.)
Anthesis (not decapitated)	Terminal	35.4±1.0
Terminal meristem vegetative	1	42.8±1.0
	9	28.3±2.9
Reproductive terminal meristem just initiated	1	40.5±1.7
	12	28.5±0.6
Inflorescence visible	5	36.6±1.9
	17	25.2±2.5
Anthesis (first flower open)	5	39.0±3.2
	17	23.2±3.1
	22	13.8±0.5

*Plants were grown from seed under standard greenhouse conditions. Decapitations were made at the internode above the bud indicated and several of the axillary buds below this bud were removed so as to promote its growth. The first node is considered to be the node which produces the first leaf that hangs over the edge of a 7-inch pot. About five or six leaves are produced before this 'first' node. Nodes were numbered acropetally with node number 1 at the base.

developmental response of a bud is a function of its position on the stem. This position-dependent response could be a function of the age of the bud, of its actual position, or of the state of differentiation of the vascular connections between the bud and the main axis.

The developmental potential of isolated buds was examined by rooting buds without the use of exogenous phytohormones, and growing them to maturity. Twenty-five buds from various nodes of several plants produced 36.1 nodes (s.d.=3), indicating that they had no 'memory' of their original position, which suggests that a bud's pattern of development may depend on information received from the plant. To examine this, we grafted buds in four patterns; apical bud to apical internode, apical bud to basal internode, basal bud to basal internode, and basal bud to apical internode (Table 2). When the 21st bud was grafted to the 22nd internode and released from apical dominance by decapitation, it produced twice as many nodes as the intact 22nd bud released from apical dominance (25.3 as against 13.8), and when grafted to the 6th internode, about three times as many (37.3). A 5th bud grafted to a 6th internode produced about the same number as an intact 5th bud (41.5 as against 39.0) but $\sim 1/3$ fewer when grafted to the 22nd internode (27.3). These results clearly show that the number of nodes produced can be altered by moving the bud up or down on the stem. The increased number of nodes produced by an apical bud that was grafted into an apical position was, however, puzzling. Since grafting involves significant injury as well as the establishment of new vascular connections, we examined the growth pattern of apical buds after severance of the vascular connections and tissues below the bud by a horizontal incision. As with grafting, this treatment resulted in an increased number of nodes (Table 2). The increased number of nodes produced is probably not primarily a response to injury, but rather reflects an altered movement of materials from the main axis to the bud and vice versa.

Our experiments have shown that the number of nodes produced by an intact axillary meristem is a function of its position on the stem. Three lines of evidence suggest that the apical meristem does not autonomously control the number of nodes produced; first, all rooted buds produce about the same number of nodes; second, grafted buds can produce more or fewer nodes than expected from the bud if it had remained intact; and third, when the tissues below a bud are cut, the bud produces more nodes than expected. Thus, the meristem seems to respond to information from the plant since the developmental pattern of a grafted bud can be altered by moving it up or down the plant. The increased number of nodes produced by grafting or cutting the tissues below an apical bud suggests, however, that appropriate transmission of this information may not occur through newly regenerated tissues. It is also possible that the temporary separation of the bud from the lower part of the plant may disrupt a positional information signal and thereby lead to an altered developmental response. In any event, the state of differentiation of the tissues connecting the bud with the main axis seems to be very important in maintaining developmental growth potential.

The observations that *N. tabacum* plants produce a uniform number of nodes in certain environmental conditions³ and that various axillary buds from a single plant can express different growth potentials^{4,5} are not unique. The experimental demonstration that the number of nodes produced by an organised meristem before commencing determinate growth is a function of the total number of nodes in the plant has not, however, been reported previously. This suggests that the terminal meristem (an axillary meristem—grafted or intact—becomes the terminal meristem after decapitation) receives information from the plant which orients the cells of the meristem in terms of their place on the main axis. This orientation in turn

Table 2 Nodes produced after grafting or wounding

Treatment	Node number of bud that developed†	No. of nodes produced by bud in column two ($\pm$ s.d.)
Grafting*		
21st bud to 22nd internode	21	25.3 $\pm$ 2.1
21st bud to 6th internode	21	37.3 $\pm$ 1.3
5th bud to 6th internode	5	41.5 $\pm$ 2.6
5th bud to 22nd internode	5	27.7 $\pm$ 0.4
Wounding		
Decapitated—not wounded	26	15.8 $\pm$ 3.8
Decapitated—wounded below node 26†	26	22.5 $\pm$ 0.6

*The bud to be grafted was removed by cutting round the bud through the vascular tissues to the pith and then lifting off the patch containing the bud. At the graft site the bud was slipped into a T-shaped cut and wrapped with parafilm for several days. After 10 d the plant was decapitated above the grafted bud.

†Plants were wounded by making a cut 1.5 cm below the bud and half way through the stem. Post-wound treatment was the same as for grafted buds.

‡Nodes were numbered acropetally with node number 1 at the base.

determines the future developmental pattern. The orientation signal might be one or more chemical gradients which change as the number of nodes increases, and at a critical value of the concentration of the chemicals, the meristem cells alter their gene expression so that floral development ensues. This idea is supported by the work of Schwabe and Al-Doori⁶ who proposed that a gibberellic acid (GA) gradient in the black currant (*Ribes nigrum*) may be responsible for the flowering response of axillary meristems. They have shown that the ability of an axillary meristem to flower in short-day photoperiods is directly related to its position on the main axis, and that this response is correlated with the GA gradient.

This regulation of pattern development may be analogous to a model of animal pattern development, where differential gene expression is thought to be intimately related to the orientation of a cell within a developing structure or organism, and that this orientation involves the reception and interpretation of a positional information signal⁷. Although the model has not often been used in studies of pattern development in plants^{1,7}, positionally-related development of shoots and leaves has been observed in numerous plants^{4,5,8,9}. These observations and the above results suggest that the use of a positional information model could introduce new ways of examining and interpreting developmental patterns in plant systems.

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CARL N. McDANIEL*
FRANCIS C. HSU

Department of Biology,
Yale University,
New Haven, Connecticut 06520

Received August 25; accepted December 18, 1975.

*Present address: Department of Biology, Rensselaer Polytechnic Institute, Troy, New York 12181.

¹ Steeves, T. A., and Sussex, I. M., *Patterns in Plant Development*, (Prentice-Hall, Englewood Cliffs, New Jersey, 1972).

² Lang, A., in *Encyclopaedia of Plant Physiology*, XV/1 (edit. by Ruhland, A.), 1380–1536 (Springer, Berlin, 1965).

³ Seltman, H., *Bot. Gaz.*, 135, 196–200 (1974).

⁴ Bancelhon, L., *C. r. hebdomadaire Séances Acad. Sci., Paris*, D262, 1228–1231 (1966).

⁵ Yeung, E. G., and Peterson, R. L., *Can. J. Bot.*, 50, 73–78 (1972).

⁶ Schwabe, W. W., and Al-Doori, A. H., *J. exp. Bot.*, 24, 969–981 (1973).

⁷ Wolpert, L., in *Curr. Top. dev. Biol.*, 6, 183–224 (1971).

⁸ Sinnott, E. W., *Plant Morphogenesis*, (McGraw-Hill, New York, 1960).

⁹ Nozeran, R., Bancelhon, L., and Neville, P., in *Advances in Morphogenesis* (edit. by Abercrombie, M., Brachet, J., and King, T. J.), 9, 1–67 (Academic, New York, 1971).

Unique Golgi apparatus and vesicle formation in a red alga

THE morphology of the Golgi apparatus, as well as the kind and quantity of Golgi-derived vesicles, has been observed to change considerably during carposporogenesis (production of diploid reproductive spores¹) in red algae^{2,3}. In *Polysiphonia* the Golgi is initially active in the production of vesicles destined for wall formation (unpublished), the same function as reported in other red algae^{2,3}. At this stage in early development, crystalline structures termed "striated vesicles" have been observed to form within rough endoplasmic reticulum (ER)^{2,4}. As carpospore development proceeds the striated vesicles diminish in size and eventually disappear (unpublished). Coinciding with this disappearance is a unique change in the form and function of the Golgi apparatus. The striated vesicles are replaced in the cytoplasm by Golgi-derived vesicles with a crystalline content, their structure and periodicity being different from the striated vesicles. The Golgi apparatus gradually differentiates into its new role, and is not replaced by a new system. Golgi with a similar morphology and function have not been observed before for any cell.

Sexual plants of *Polysiphonia novae-angliae* Taylor were collected in July 1972, at Naushon Island, and in June 1973, at West Falmouth, Massachusetts. The methods used to prepare for electron microscopy have been described before⁴. Figure 1 shows completely differentiated Golgi active in the formation of vesicles with a crystalline content. Mitochondria (m, Fig. 1a) are observed at the base of the forming Golgi face, a situation which is typical throughout carpospore differentiation^{2,3}. Proximal cisternae arise from the fusion of ER-derived vesicles which contain a dark osmiophilic material (arrows, Fig. 1c). The periphery of proximal cisternae are swollen with dark osmiophilic regions surrounded by a less dense material (a, Fig. 1a-c). As cisternae mature and are displaced towards the distal Golgi face, the material surrounding the dark osmiophilic regions becomes fibrous with a distinctive pattern and spacing (b, Fig. 1a-c). The osmiophilic regions are not continuous, but somewhat spherical and periodically spaced within a single cisterna. Therefore, they may appear absent in some sections. The fibrous structure gives way to a dark grey, uniformly stained vesicle at the distal pole (c, Fig. 1a and b). The osmiophilic regions then disappear (d, Fig. 1b) as the cut off vesicles take on a fibrous crystalline pattern (e, Fig. 1a, b and d). Ribosomes are not observed to be associated with the membrane surrounding these Golgi-derived vesicles. The vesicles remain in the cytoplasm for a short period in development and then gradually disappear. At this time the Golgi apparatus once again changes in form and function, producing yet another kind of vesicle (unpublished).

It is interesting that the change in Golgi function reported here corresponds exactly with the disappearance of the striated vesicles formed by the rough ER. There may be some correlation between this disappearance and the concurring Golgi differentiation. The dark osmiophilic regions seen in developing cisternae arise during the formation of cisternae, the material having the same appearance as the contents of the fusing ER vesicles at the proximal face. As the periphery of the maturing cisternae enlarge, most contents undergo structural change whereas the osmiophilic regions seem unaltered until near maturity. Contents therefore seem segregated in individual developing cisternae, one transferred from the ER, the other synthesised within the Golgi. Only in mature vesicles do the contents become uniform. Several different types of

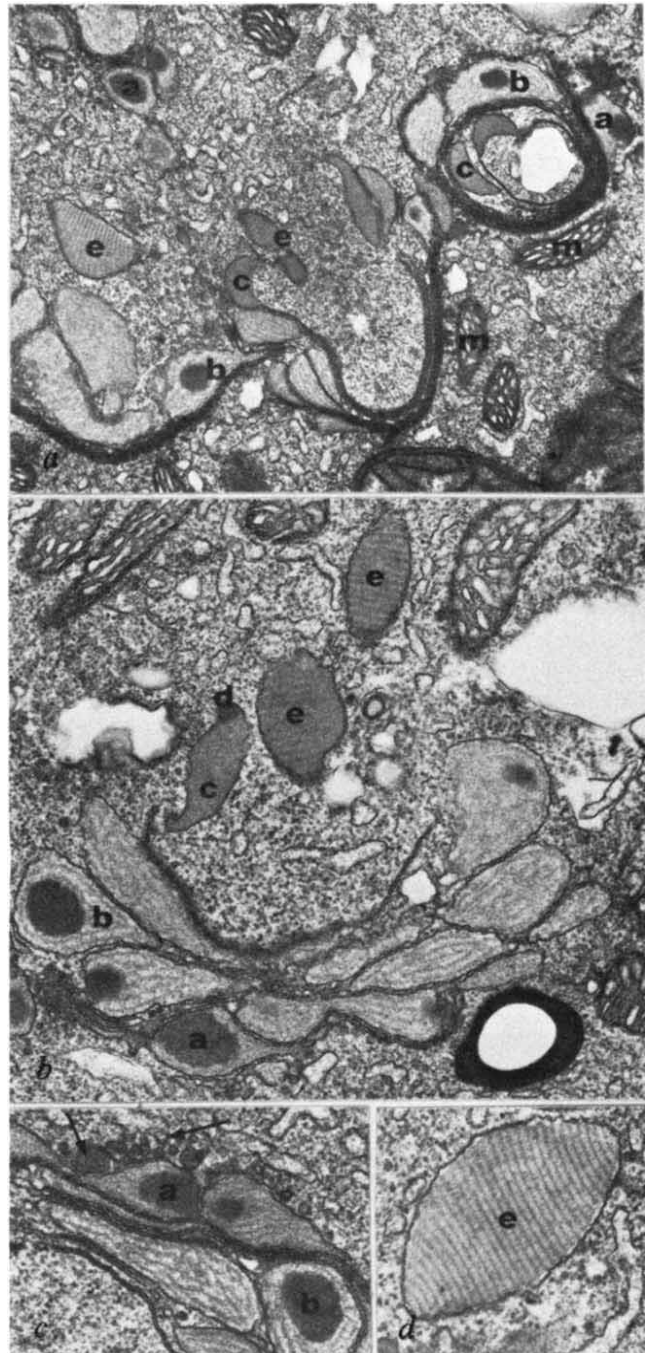


Fig. 1 a, Mature Golgi apparatus active in the production of crystalline vesicles. Mitochondria (m) are located at the base of the proximal Golgi face when sectioned through that region. a, Initial stage in maturation of cisternae. Note the dark osmiophilic region surrounded by a less dense material. b, Stage in maturation of cisternae. Osmiophilic regions are surrounded by a fibrous material. c, Vesicle just before crystalline structure arises. d, Osmiophilic region disappearing in mature vesicle. e, Separated vesicles with crystalline structure. ($\times 15,390$). b, Glancing section of mature Golgi showing the different stages of cisternal development ($\times 27,060$). c, ER-derived vesicles (arrows) active in cisterna formation ($\times 25,080$). d, Structure of crystalline vesicle ($\times 31,900$).

secretory cells are known to use the Golgi apparatus for the concentration and transport of protein to the exterior of the cell⁵⁻⁷. The combination of protein with other products during this process has likewise been suggested^{6,7}. Crystalline structures described within other cells have consistently been determined to be at least partially proteinaceous^{9,10}. This is the first observation of Golgi-derived vesicles with a crystalline content; since these vesicles are

retained in the cytoplasm only for a short period during development, evidence suggests that the Golgi has a role in the transformation and turnover of protein within differentiating carpospores. Golgi apparatus with a similar fine structure and function have not been reported before.

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RICHARD WETHERBEE

School of Botany,
University of Melbourne,
Parkville 3052, Victoria,
Australia

JOHN A. WEST

Department of Botany,
University of California,
Berkeley, California 94720

Received October 14, 1975; accepted January 2, 1976.

- ¹ Iyengar, M. O. P., and Balakrishnan, M. S., *Proc. Indian. Acad. Sci.*, **29**, 105 (1949).
- ² Kugrens, P., and West, J. A., *Phycologia*, **12**, 163 (1973).
- ³ Hawkins, E. K., *Protoplasma*, **80**, 1 (1974).
- ⁴ Wetherbee, R., and Wynne, M. J., *J. Phycol.*, **9**, 402 (1973).
- ⁵ Jamieson, J. D., and Palade, G. E., *Proc. natn. Acad. Sci. U.S.A.*, **55**, 422 (1966).
- ⁶ Leblond, C. P., in *The Use of Radioautography in Investigating Protein Synthesis* (edit. by Leblond, C. P., and Warren, K. B.), 321–339 (Academic, New York, 1965).
- ⁷ Seed, R. W., and Goldberg, I. H., *Proc. natn. Acad. Sci. U.S.A.*, **50**, 275 (1963).
- ⁸ Morre, D. J., Mollenhauer, H. H., and Bracker, C. E., in *Origin and Continuity of Cell Organelles* (edit. by Reinert, J., and Ursprung, H.), 82–126 (Springer, New York, 1971).
- ⁹ Chaley, N. M., Haggis, G. H., and Setterfield, S., *J. ultrastruct. Res.*, **49**, 321 (1974).
- ¹⁰ Wood, D. O., and Luck, D. J. L., *J. Cell Biol.*, **51**, 249 (1971).

Possible regeneration of γ -aminobutyric acid-containing fibres into irides transplanted into the central nervous system

IRIDES transplanted into the central nervous system (CNS) of the rat provide a good substrate for regenerative ingrowth of central noradrenergic and cholinergic fibres to 'reinnervate' the denervated iris^{1–3}. Fluorescence histochemistry or acetylcholinesterase staining reveals that the regenerating noradrenergic or cholinergic fibres will produce an innervation that closely mimics the original noradrenergic or cholinergic innervation^{1–3}. Physiological experiments⁴ on portal vein transplants indicate that functional adrenergic and cholinergic terminals are formed, and it seems likely that functional neuromuscular junctions are also re-established in the iris transplants. In addition to supporting the ingrowth of noradrenergic and cholinergic fibres, fluorescence histochemistry² reveals that 5-hydroxy-tryptamine- and dopamine-containing fibres will also grow into iris transplants, albeit less extensively than noradrenergic fibres. This ability of the iris to support regenerative ingrowth by fibres not entirely appropriate for normal reinnervation suggested to us that the iris might also support regenerative ingrowth by fibres containing putative amino acid transmitters.

As a possible marker for γ -aminobutyric acid (GABA) terminals we choose to measure the GABA synthetic enzyme, glutamic acid decarboxylase (GAD). GAD is located in nerve terminals and is concentrated in areas of the brain where GABA is believed to act as a neurotransmitter^{5,6}. We demonstrate here that irides transplanted into the adult rat CNS show increasing levels of glutamic acid decarboxylase consistent with the ingrowth of GAD containing fibres. In addition, using a microdansylation assay⁷ we have measured the more concentrated amino acids in the transplants and in normal and sympathectomised irides, and found substantial increases in levels of GABA, glutamate and glutamine in the transplants, which would be consistent with regenerative ingrowth of fibres

containing relatively high concentrations of these amino acids.

The experiments were carried out on adult female Sprague-Dawley rats (180–200 g body weight). Autologous transplantations of the iris were made in two brain sites; one in the hippocampal fimbria and the rostral hippocampus (CA3), as described earlier³, and a second in the head of the caudate nucleus (unpublished). In both sites the transplants survive well with little bleeding and become reinnervated by acetylcholinesterase-containing fibres, and in the caudate site by dopamine-containing fibres as well. All animals were subjected to cranial sympathectomy through bilateral extirpation of the superior cervical ganglia. In addition, as a control site, irides were transplanted to the anterior eye chamber⁸. In one experiment irides transplanted to the caudate nucleus 21 d earlier were transplanted to the anterior eye chamber. Here, they were allowed to survive for another 4 d. GAD levels were initially measured by ¹⁴CO₂ evolution^{9,10} in the presence of 0.5% Triton X-100, a concentration recently shown¹¹ to reduce the possible formation of ¹⁴CO₂ by glutamate entering the tricarboxylic acid cycle as a ketoglutarate.

The results are summarised in Fig. 1, and the assay conditions given in more detail in Table 1. In both the caudate nucleus and the fimbrial-hippocampal site there was a marked increase in GAD in the transplants, its appearance following a time development similar to that of the in-

Table 1 Comparison of glutamic acid decarboxylase activity in normal and sympathectomised irides, and in irides transplanted to caudate and fimbrial sites

	GABA production (nmol per mg protein per h)	¹⁴ CO ₂ production (nmol per mg protein per h)
Normal iris	< 0.080 (3)	2.38 ± 0.6 (12)
Sympathectomised iris	< 0.080 (3)	3.38 ± 0.7 (6)
Iris transplant to anterior eye chamber (26 d after transplantation)	—	2.7 ± 1.7 (6)
Caudate transplant (21 d GABA production, or 26 d ¹⁴ CO ₂ production)	4.47 ± 1.16 (5)**	14.9 ± 1.2 (6)*
Fimbrial transplant (21 d GABA production or 14 d ¹⁴ CO ₂ production)	4.23 ± 0.67 (3)**	10.5 ± 1.6 (6)*

Values are means ± s.d. Means were derived by Student's *t* test (two tailed) **P* < 0.05, ***P* < 0.01. The figures in parentheses are numbers of replicates. ¹⁴CO₂ production was measured using the micromodification of the method of Albers and Brady⁹ described by Fonnum *et al.*¹⁰. DL-1-¹⁴C-glutamate (58 mCi mmol⁻¹, New England Nuclear) was used as substrate. 2 µl of an iris homogenate (≈ 1.0 mg per 20 µl buffer) was incubated for 1 h with a substrate containing final concentrations: L-1-¹⁴C-glutamate, 0.30 mM; K-L-glutamate, 4.03 mM; potassium phosphate buffer (pH 6.5), 40.3 mM; pyridoxal-5-phosphate, 0.260 mM; dithiothreitol, 2.38 mM and 0.5% Triton X-100. ¹⁴CO₂ was evolved by adding a drop of 1 M sulphuric acid and ¹⁴CO₂ collected in a separate microtube containing 50 µl solene (Packard). ¹⁴C-GABA production was measured using L-(U)-¹⁴C-glutamate (290 mCi mmol⁻¹, Radiochemical Centre, Amersham). 10 µl of iris homogenate (≈ 1.0 mg per 10 µl) was incubated for 3 h with a substrate containing final concentrations: L-(U)-¹⁴C-glutamate, 0.17 mM; K-L-glutamate, 3.4 mM; potassium phosphate buffer (pH 6.5), 34 mM; pyridoxal-5-phosphate, 0.44 mM; dithiothreitol, 3.9 mM; 0.25% Triton X-100 and 1.35 µM amino-oxyacetic acid. The reaction was stopped by addition of 0.96 N perchloric acid, the protein centrifuged down and the pellet used for protein determination by the method of Lowry *et al.*¹³. The supernatant (20 µl) after neutralisation with 2 M potassium carbonate was reacted with 20 µl dansyl chloride (2 mg ml⁻¹) (British Drug Houses) for 2 h, evaporated to dryness and taken up in 20 µl acetone-acetic acid (3:2, v/v). The total volume was applied to full size polyamide plates and dansyl GABA separated by chromatography as described by Joseph and Haliday⁷. ¹⁴C-GABA standards were taken through the procedure to correct for recovery. There was no spread of the labelled substrate (dansyl glutamate) to contaminate the dansyl GABA spot and no significant radioactivity was detected outside these spots. The limit of sensitivity of this assay, double the blank value, was equivalent to ≈ 0.25 pmol.

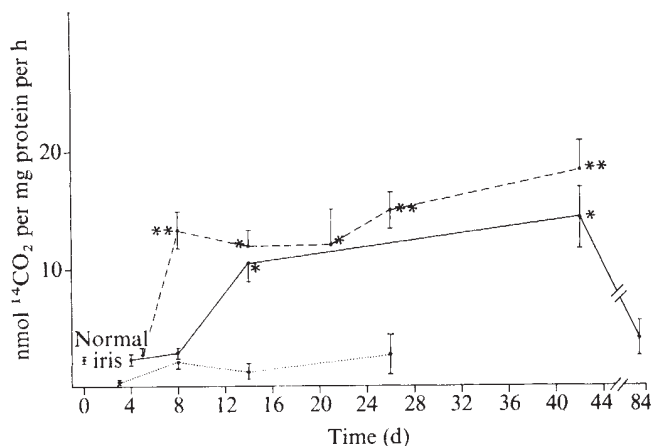


Fig. 1 Glutamic acid decarboxylase activity of irides transplanted to a fimbrial-hippocampal site (—), a caudate site (---) and an ocular site (....). Values are means $\pm$ σ of at least five separate determinations at each time point. Differences from normal iris were compared using Student's *t* test (two tailed) **P* < 0.05, ***P* < 0.01.

growth of regenerating monoaminergic and cholinergic fibres (see refs 2, 3 and unpublished observations). The increase in GAD cannot be a response to denervation alone, as sympathectomy (Table 1) does not significantly alter the level of GAD in the iris. Moreover, the increase in GAD does not occur after transplantation of the iris to the anterior eye chamber (Fig. 1). In fact, there seemed to be a temporary decrease in GAD levels suggesting that some of the GAD activity of the normal iris might reside in the muscle coat which, during the early stages after transplantation, undergoes degeneration¹².

The reduction in $^{14}\text{CO}_2$ production seen in the 4-d-old irides transplanted to the anterior eye chamber indicates the problems of correlating $^{14}\text{CO}_2$ evolution with glutamic acid decarboxylase activity. For this reason we felt it necessary to also measure GAD by GABA production from L-(U)- ^{14}C -glutamate (Table 1). The results show that in both the caudate and the fimbrial-hippocampal sites definite GABA formation occurred in the transplanted irides, whereas GAD activity was low or absent in the normal or sympathectomised irides. Measured in this way there was a 100-fold increase in GAD activity in the CNS implants over the normal or sympathectomised irides. The

average GAD activity of the transplants measured by ^{14}C -GABA formation was, however, less than that observed with the CO_2 evolution assay. These differences were not great and could readily be explained by the normal variations in GAD activity of the transplants and by the fact that the GAD assay is undoubtedly no longer linear after 3 h incubation. Three hours was chosen to ensure sufficient counts in the dansyl-GABA spot; the apparent GAD activity would be greater if the incubation were performed over 1 h. The very small amount of GABA formed in the normal iris (in some cases no formation was detected) confirms our suggestion that basal level of GAD activity in the normal iris as measured by $^{14}\text{CO}_2$ production is not related to GABA formation but rather probably represents glutamate metabolism by other pathways to yield $^{14}\text{CO}_2$.

In addition to demonstrating the appearance of significant levels of GAD in the transplanted irides it was of great interest to look at changes in the levels of amino acids in the irides after transplantation to the two brain sites (Table 2). In both the caudate and fimbrial-hippocampal sites there were significant increases in GABA, glutamate and glutamine above the levels in normal or sympathectomised irides. In contrast, the levels of taurine, glycine and aspartate did not change significantly. The level of GABA (2.3–2.5 μmol per g protein) correlates well with the level of GAD (20–30 μmol per h per g protein) and the ratio of GABA–GAD is similar to that found in the caudate nucleus or hippocampus and other sites believed to contain GABA terminals^{14,15}. Glutamine may have an important role in GABA metabolism or ammonia formation¹⁶. It seems possible, therefore, that the appearance of GABA and glutamine in the central implants both reflect the ingrowth of GABA-ergic fibres. This might be the case with glutamate as well, but it also seems possible that glutamate could be found in a separate, glutamate-rich fibre type (see refs 15, 17–20).

These various results are consistent with a growth into the transplanted irides of GABA-synthesising and GABA-containing elements from the brain tissue surrounding the transplantation site. There are two possible, though unlikely, objections to this interpretation. First, the increased GAD and amino acid levels could arise from a growth of fragments of retinal tissue (which contain both GAD and GABA) which might be attached to the iris transplant. This can be excluded, however, since adult retinal tissue does not survive transplantation to the CNS sites used

Table 2 Amino acid levels in transplants from fimbrial-hippocampal and caudate sites (21 d post transplantation)

Amino acid	Normal iris (<i>n</i> = 3)	Sympathectomised iris (<i>n</i> = 3)	Caudate transplant (<i>n</i> = 5)	Fimbrial-hippocampal transplant (<i>n</i> = 4)	Caudate nucleus (<i>n</i> = 3)	Hippocampus (<i>n</i> = 4)
γ -Aminobutyric acid	0.02 $\pm$ 0.005	0.030 $\pm$ 0.014	0.23 $\pm$ 0.012†(‡)	0.25 $\pm$ 0.042†(‡)	1.85 $\pm$ 0.24	1.76 $\pm$ 0.13
Glycine	0.35 $\pm$ 0.052	0.52 $\pm$ 0.03*	0.50 $\pm$ 0.03	0.61 $\pm$ 0.10	0.36 $\pm$ 0.02	0.42 $\pm$ 0.021
Glutamine	0.307 $\pm$ 0.077	0.373 $\pm$ 0.13	2.68 $\pm$ 0.35†(‡)	3.35 $\pm$ 1.24(*)	4.59 $\pm$ 0.29	4.90 $\pm$ 0.55
Glutamate	0.501 $\pm$ 0.022	0.68 $\pm$ 0.09	1.26 $\pm$ 0.078†(‡)	1.40 $\pm$ 0.15†(‡)	5.08 $\pm$ 0.126	6.16 $\pm$ 0.35
Aspartate	0.71 $\pm$ 0.037	0.473 $\pm$ 0.09	0.59 $\pm$ 0.083	0.97 $\pm$ 0.34	0.97 $\pm$ 0.16	0.73 $\pm$ 0.11
Taurine	4.01 $\pm$ 0.87	4.22 $\pm$ 0.54	3.41 $\pm$ 0.4	4.0 $\pm$ 0.87	5.82 $\pm$ 0.25	4.75 $\pm$ 0.28

Levels in transplants are compared with amino acid levels in the normal and sympathectomised iris and with levels in the caudate nucleus and hippocampus. Units are μmol per 100 mg protein.

Means were compared with Student's *t* test (two tailed) **P* < 0.05, †*P* < 0.01, ‡*P* < 0.001. () indicate significance if normal and sympathectomised iris data are pooled for comparison with fimbrial and caudate transplants. Amino acid levels were determined using the microdialysis assay for amino acids described in detail by Joseph and Haliday⁷. Because of the small amounts of tissue in the iris samples (0.8–1.5 mg wet weight) the samples were homogenised in 10 μl of 0.48 M perchloric acid, and after centrifuging 2 μl of the ^{14}C -amino acid mixture (GABA, glycine, glutamine, glutamate and aspartate, Radiochemical Centre, Amersham) and 2 μl 2 M potassium carbonate were added to the supernatant and the sample again centrifuged to remove the precipitated perchlorate. An aliquot of the supernatant (2 μl) was reacted with ^3H -dansyl chloride (5.1 mCi μmol^{-1} , Radiochemical Centre, Amersham) for 30 min at room temperature after which the sample was evaporated to dryness. The dansylated sample was redissolved in 5 μl acetone–acetic acid (3:2, v/v) and 0.3 μl applied to small polyamide plates 3.5 cm². The dansylated amino acids were separated using the solvents described by Joseph and Haliday⁷, the spots visualised, cut out and the ^3H – ^{14}C ratio determined by two-channel liquid scintillation spectrometry. The ^3H – ^{14}C ratio is independent of recovery and degree of dansylation. Suitable standards and blocks are run simultaneously and the amount of amino acid determined from standard values. Protein content of the pellets was determined by the method of Lowry *et al.*¹³.

Table 3 Effects of chloride ions (as sodium chloride) on apparent GAD activity in irides transplanted into caudate nucleus (21 d survival), in control caudate nucleus tissue, and in tissue from the pineal gland

	No chloride in incubation mix (GAD)	200 mM chloride in incubation mix (GAD)	Enzyme assayed	Irides transplanted from caudate nucleus (21 d survival) to anterior eye chamber (4 d survival)	Irides transplanted to caudate nucleus (26 d survival)
Irides transplanted to caudate nucleus (21 d survival)	12±3.2	6.1±3.4*	GAD	ND	14.9±1.2
Pineal gland	9.3±1.1	8.9±1.0	ChAT	< 0.14(*)	89±10
Caudate nucleus	223±14	129±21*	AAD	ND	5.9±0.4

The table also shows the effects of retransplantation on GAD activity, choline acetyltransferase activity (ChAT) and aromatic amino acid decarboxylase activity (AAD) in irides transplanted to the caudate nucleus and then retransplanted into the anterior eye chamber of a different rat. For comparison, enzyme levels from iris transplants removed from the caudate nucleus site after 26 d and then assayed for ChAT, AAD and GAD are shown (this paper and Emson, Björklund and Stenevi, unpublished). Values are means $\pm$ s.d. Units are nmol per mg protein per h. Means were compared using Student's *t* test (two tailed) on paired or unpaired samples. **P* < 0.001, (*) paired *t* test, *P* < 0.001. ND = not detectable.

(unpublished observations), and since identical transplants to the anterior eye chamber (which is a comparable or even better transplantation site than the brain) did not show the increased GAD levels. Second, it might be argued that, in removing the iris transplants from the brain, we are also removing trace amounts of surrounding tissue rich in GAD and amino acids. We were aware of this possibility and paid particular attention during dissection to removing all brain tissue fragments visible under high magnification in the dissection microscope. Random identical specimens were also taken for microscopic analysis showing that the amount of adhering tissue was minimal, at least up to ~ 1 month after transplantation. The amount of tissue adhering would, moreover, need to be fairly substantial to account for the GAD levels observed. The GAD level in the caudate nucleus is 217 μ mol per h per g protein¹⁴, whereas the GAD activity in the best caudate implants was 30–40 μ mol per h per g protein. To account for the weight GAD activity in a transplant (~ 0.8 mg wet weight) we would need $\sim 100 \mu$ g of adhering tissue, an amount we would be most unlikely to fail to detect. It is also obvious that if adhering tissue would account for the observed increases in amino acid levels in the transplants, then the increases should reflect the relative abundance of the various amino acids in the tissue surrounding the transplant. This was not the case (see Table 2).

Thus, it seems likely that the observed increases in enzyme and amino acid levels in the centrally transplanted irides resulted from the growth of tissue elements from the surrounding, lesioned brain tissue. To the extent that GAD and the GABA–GAD ratio can be taken as good markers for GABA-containing terminals in the brain, the GAD and GABA levels in the implanted tissue should provide good measures of the sprouting of regenerating GABA-ergic fibres into the implant from the lesioned brain tissue. The likely origin of such sprouting GABA- and GAD-containing fibres would in both the caudate and the hippocampal sites seem to be the local GABA-ergic interneurons which have been demonstrated in these brain regions^{21,22}.

The most obvious difficulty in the interpretation of the present findings is to evaluate the contribution of glial cells to the observed increases in enzyme and amino acid levels in the transplanted irides. The strong increase in glutamine in the transplants might be an indication for this, since this amino acid may be relatively concentrated in glial cells¹⁶. Also GAD is known to occur in glial cells¹⁶, and we could not exclude the possibility that the GAD (as well as the GABA and glutamate) we measure is located at least partly in glial cells. To obtain further evidence of the neuronal localisation of the GAD activity, we carried out an experiment designed to effect a denervation of the reinnervated transplant. Irides were transplanted to the caudate nucleus as above, and allowed to survive 21 d.

After 21 d, when the GAD levels in the transplant (as measured by the CO₂ evolution assay) were near maximal, the animals were killed, and the transplants dissected out from the caudate nucleus. The iris was then retransplanted into the anterior eye chamber of another rat. Here, the iris remained for 4 more days—a survival time that would allow for the degeneration of fibres that had previously regenerated into the transplant—and was then assayed for GAD, choline acetyltransferase (ChAT) and aromatic amino acid decarboxylase (AAD). As shown in Table 3 there was a complete or almost complete loss of all three enzymes. These results are consistent with a degeneration of cholinergic, dopaminergic and GABA containing terminals that had grown into the transplant while being in the caudate nucleus. A further pointer to the probable neuronal origin of the GAD in the transplant is shown by its response to Cl[−] ions²³. GAD activity in the pineal gland, which is believed to be exclusively glial in origin, was not significantly reduced by the addition of Cl[−] ions, whereas the activity in the transplants—like that in the caudate nucleus—was reduced some 50% (*P* < 0.001).

Very little is known about the regeneration of GABA neurons. Baxter and Torralba^{24,25} have provided some evidence for regeneration of GABA neurons in insects. Recent studies (see refs 2 and 3) have shown that many non-myelinated axon systems in the adult mammalian CNS have a high capacity for regenerative sprouting. The present results are compatible with the idea that this is the case also for non-myelinated GABA-ergic interneurons in the hippocampus and the caudate nucleus. If this is so our observations constitute the first direct evidence for regeneration of GABA neurones in the adult mammalian CNS.

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P. C. EMSON*

*Beit Memorial Fellow,
MRC Brain Metabolism Unit,
1 George Square, Edinburgh EH8 9JZ, UK*

ANDERS BJÖRKLUND
ULF STENEVI

*Department of Histology,
University of Lund,
Biskopsgatan 5, S-223 62 Lund, Sweden*

Received October 10; accepted December 3, 1975.

*Present address: MRC Neurochemical Pharmacology Unit, Hills Road, Cambridge, UK.

¹ Björklund, A., and Stenevi, U., *Brain Res.*, **31**, 1 (1971).

² Svendgaard, N. A., Björklund, A., and Stenevi, U., *Adv. Anat. Embryol. Cell Biol.*, **51**, 1 (1975).

- ³ Svendgaard, N. A., Björklund, A., and Stenevi, U., *Brain Res.*, **102**, 1 (1976).
⁴ Björklund, A., Johansson, B., Stenevi, U., and Svendgaard, N. A., *Nature*, **253**, 446 (1975).
⁵ Fonnum, F., *Biochem. J.*, **106**, 401 (1968).
⁶ Fonnum, F., and Walberg, F., *Brain Res.*, **62**, 577 (1973).
⁷ Joseph, M. H., and Haliday, J., *Analyt. Biochem.*, **64**, 389 (1975).
⁸ Olson, L., and Malmfors, T., *Acta physiol. scand. suppl.*, **348** (1970).
⁹ Albers, R. W., and Brady, R. O., *J. Biol. Chem.*, **234**, 926 (1959).
¹⁰ Fonnum, F., Storm-Mathisen, J., and Walberg, F., *Brain Res.*, **20**, 259 (1970).
¹¹ MacDonnell, P., and Greengard, O., *J. Neurochem.*, **24**, 615 (1975).
¹² Campbell, G. R., Uehara, Y., Malmfors, T., and Burnstock, G., *Z. Zellforsch.*, **117**, 155 (1971).
¹³ Lowry, O. H., Rosenbrough, N. H., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 65 (1951).
¹⁴ Storm-Mathisen, J., *Brain Res.*, **87**, 107 (1975).
¹⁵ Emson, P. C., and Joseph, M. H., *Brain Res.*, **93**, 91 (1975).
¹⁶ Minchin, M. E. W., and Beart, P. M., *J. Neurochem.*, **24**, 881 (1975).
¹⁷ Riefenstein, R. J., and Neal, M. J., *Can. J. Physiol. Pharmacol.*, **52**, 667 (1974).
¹⁸ Harvey, J. A., Scholfield, C. N., Graham, L. T., Jr., and Aprison, M. H., *J. Neurochem.*, **24**, 445 (1975).
¹⁹ Synder, S. H., Young, A. B., Bennet, J. P., and Mulder, A. H., *Fedn Proc.*, **32**, 2039 (1974).
²⁰ Curtis, D. R., et al., *Brain Res.*, **41**, 283 (1972).
²¹ Storm-Mathisen, J., and Fonnum, F., *Progr. Brain Res.*, **36**, 40 (1972).
²² Kataoka, K., Bak, I., Hassler, R., Kim, J. S., and Wagner, A., *Expl Brain Res.*, **19**, 217 (1972).
²³ Haber, E., Kuriyama, K., and Roberts, E., *Biochem. Pharmacol.*, **19**, 1119 (1970).
²⁴ Baxter, C. F., and Torralba, G. F., *Proc. 3rd Int. Meeting Int. Soc. Neurochem.* (Budapest), 337 (1971).
²⁵ Baxter, C. F., and Torralba, G. F., *Brain Res.*, **84**, 383 (1975).

Absence of transient tritanopia after adaptation to very intense yellow light

STILES reported¹ that after the eye had been adapted to light of high intensity and long wavelength, its sensitivity to violet test flashes did not recover according to the familiar dark-adaptation curve. Instead the threshold intensity for detecting short-wavelength flashes increased when the adapting field was turned off, and the peak sensitivity of the eye lay at long wavelengths. Yet it was by using very similar conditions that Auerbach and Wald² isolated a photoreceptor with peak sensitivity in the violet. This contradiction has not been resolved. Transient tritanopia (as we have provisionally termed the loss of sensitivity found by Stiles) has been reported by others³⁻⁶ and an electrophysiological counterpart has been described⁷; but equally there are findings that resemble the contrasting result of Auerbach and Wald^{8,9}. Attempting to resolve this contradiction, we have discovered a second surprising property of transient tritanopia.

Although our previous measurements had shown that the phenomenon occurred over a large range of adaptation intensities, and became more marked as the intensity of the adaptation field increased¹⁰, we had not used fields quite as intense as the 6.0–7.0 log trolands used by those who had found that adaptation left the eye blue-sensitive, rather than blue-blind. Moreover, the suprathreshold hue-shift described previously⁵ does not occur if the adapting field is too bright. Stiles worked with a red field of 4.3 log trolands. We have therefore measured sensitivity to short wavelengths after adaptation to retinal illuminances that varied over the range 1.1–6.0 log trolands.

Blue (445 nm) test flashes were presented 400 ms after extinction of a yellow adapting field. Target flashes subtended 1° of visual angle, were presented to the fovea and lasted 15 ms. To secure very intense adapting fields, the interference filter previously used in the adapting beam⁵ was replaced by a gelatin spectral filter (Ilford No. 626) which has a peak transmission at 575 nm and a bandwidth at half height of 35 nm. The test flashes and the adapting field were concentric and were presented in Maxwellian view. Fixation was guided by four illuminated points that were arranged in a diamond with a vertical and horizontal separation of 3° and were adjusted in intensity so that they were just clearly visible against a particular adaptation field. For 3 s every 18 s the adapting field was interrupted by a dark interval and a single target flash was presented 400 ms after the field had been turned off. (We chose 400 ms because at this delay the brief increase in threshold found for white light¹¹ has ceased, but transient tritanopia is still marked⁵.) The experiment was under computer

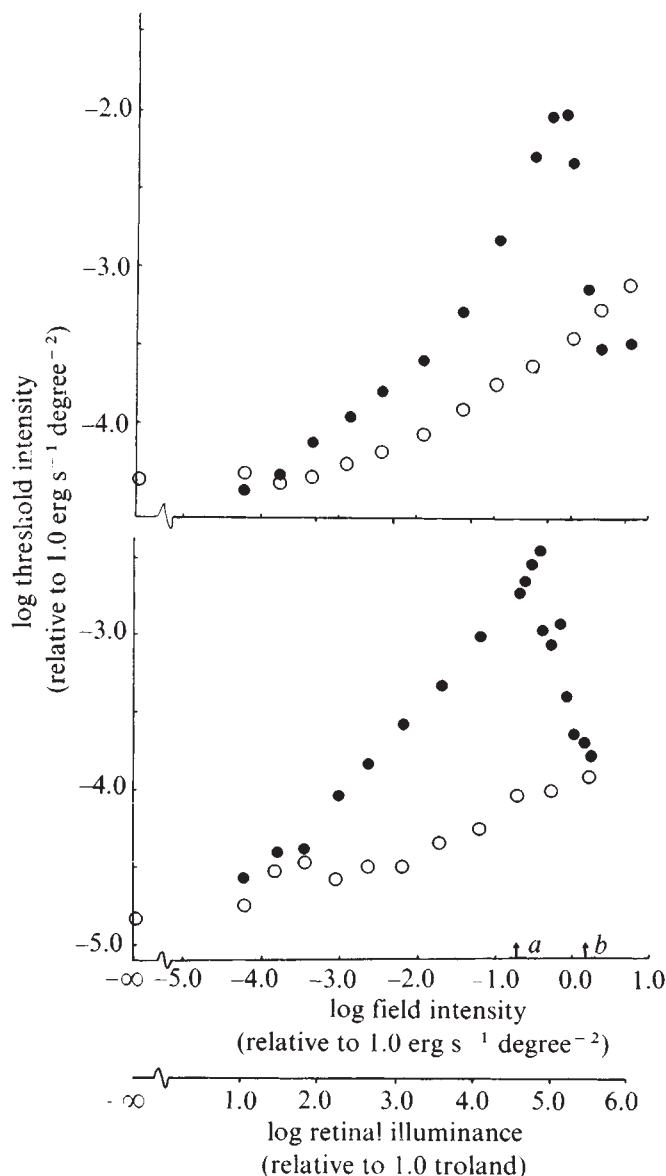


Fig. 1 Ordinate, threshold intensity at which blue target detected; abscissa, intensity of adapting field. ○, Log threshold intensity for detecting blue flash when a steady yellow field is present (conventional increment-threshold function); ●, threshold 400 ms after adapting field has been turned off (means of two runs). Field intensity marked *a* corresponds to adaptation level that gave results of Fig. 2; that marked *b* corresponds to level that gave results of Fig. 3. Observers: I.D.M. (upper panel), P.G.P. (lower panel).

control and the threshold for detecting the flash on 50% of trials was measured by a double staircase procedure¹². No data were gathered until the field had been cycling on and off for 4 min. Each estimate of the threshold was based on 50 trials.

Figure 1 shows results for two observers. When the field was present and was between 2.0 and 4.5 log trolands the mechanism responsible for detection was that termed π_1 by Stiles: note that transient tritanopia occurred even for fields that did not significantly adapt π_1 in the steady state. As the field increased to 5.0 log trolands there was an increasing difference between the threshold measured in the steady state and that measured 400 ms after the adapting field had been turned off. As Fig. 2 shows, however, the mechanism mediating detection of the flashes after extinction of a field of 4.58 log trolands had the spectral sensitivity of π_1 , Stiles' green-sensitive mechanism, and thus the true extent of the suppression of the blue cone mechanism is concealed: in this range of adaptation illu-

minance the theoretical threshold for π_1 must lie above the solid circles of Fig. 1.

The most unexpected results were found at an adaptation illuminance of about 5.0 log trolands. As the field that was turned off became brighter, the threshold fell precipitously. In other words, the eye became more sensitive to short wavelengths as the luminance of an adapting field was increased. Moreover, it was now a blue-sensitive mechanism that became responsible for detection of the 445-nm targets. Figure 3 shows the spectral sensitivity of the eye 400 ms after a field of 5.47 log trolands has been turned off; this can be compared with Auerbach and Wald's Fig. 4 (ref. 2) and contrasted with our Fig. 2.

It now seems clear why there are two apparently contradictory reports in the literature. Stiles¹ used an adapting field of 4.3 log trolands whereas those who did not report transient tritanopia^{2,8,9} used more intense fields. (The experiment of Norren and Padmos⁸ has a second relevant feature: an initial white bleaching field was replaced by an intense long wavelength field designed to preserve the light adaptation of the red- and green-sensitive mechanism while the recovery of the blue-sensitive cones was followed. If transient tritanopia arises when a recovering long-wavelength mechanism inhibits or masks the signals from the blue-sensitive cones³, we should not expect the phenomenon in the conditions used by Norren and Padmos.) A difficulty for this resolution is posed by the results of Das⁹, who reported transient tritanopia after adaptation to a field of " 1.86×10^6 trolands"; but there is a discrepancy between this value and the abscissa of his Fig. 1.

The recovery of sensitivity at adaptation illuminances above 5.0 log trolands is sudden: in the case of both observers the function relating threshold to adaptation illuminance has a maximum slope steeper than -2 . Although little is known about this phenomenon, the recovery looks like a disinhibition and a working hypothesis might be that the point at which the threshold collapses represents the retinal illuminance at which some mech-

Fig. 2 Sensitivity of eye to short wavelengths 400 ms after a yellow adapting field of 4.58 log trolands has been turned off. Ordinate, reciprocal of intensity required to detect a test flash of varying wavelength. Conditions as for main experiment, except that several test wavelengths and only one adaptation intensity were used. Observer: P.G.P. Solid curve, Stiles' field sensitivity function for π_4 displaced vertically to give best fit to experimental points.

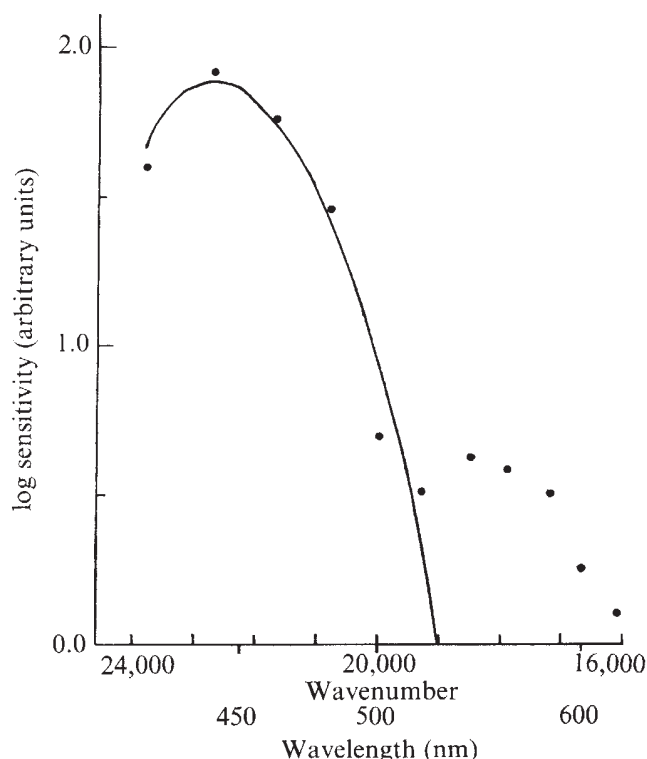
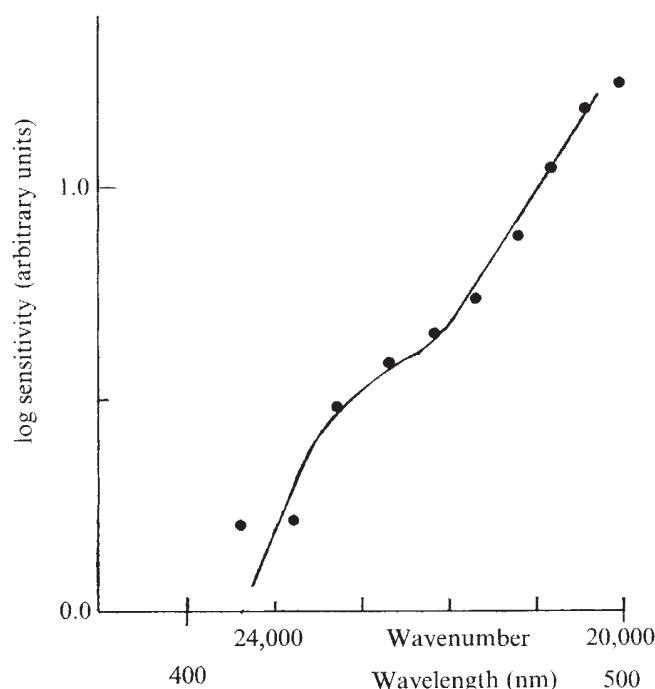


Fig. 3 Spectral sensitivity of eye 400 ms after extinction of a yellow field of 5.47 log trolands. Ordinate, reciprocal of intensity required to detect a test flash of varying wavelength. Observer: P.G.P. Solid function fitted to short wavelength points is Stiles' field sensitivity function for π_1 .

anism more sensitive to the long wavelength field has become too bleached, or otherwise too refractory, to inhibit or mask the blue-sensitive mechanism during early dark adaptation. Thus our results add to the suspicion³ that transient tritanopia represents not a direct effect of the extinction of the field on the blue cones but the inhibition or masking of the signals of the blue cones by a mechanism with different spectral sensitivity. The collapse of transient tritanopia in Fig. 1 occurs at approximately the field intensity at which the mechanism termed π_3 by Stiles replaces π_1 in the normal increment-threshold function: the inhibition seen in transient tritanopia is possibly related to an inhibition that marks the difference between π_1 and π_3 in the steady state. ' π_1 ' may represent those conditions in which the gain of the blue-sensitive mechanism is controlled by a long wavelength mechanism as well as by quanta absorbed directly by the blue-sensitive pigment.

Fields of $\sim 10^6$ trolands bleach a substantial fraction of the rhodopsin in the retina within 30 s (ref. 13) and prolonged observation of such fields (for example, in making the measurements of Fig. 3) left us with after-images that lasted for as long as 7 d. Stiles has told us that he noticed such after-images when working in similar conditions, and Brindley¹⁴ reported an after-image lasting 8 months, which was the cumulative result of repeated adaptation to high intensities. Although we can ourselves detect no permanent damage, we recommend caution in repeating our measurements: only one eye should be used and the possible dangers should be explained to all subjects.

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J. D. MOLLON
P. G. POLDEN

Department of Experimental Psychology,
Downing Street,
Cambridge CB2 3EB, UK

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- ¹ Stiles, W. S., *Doc. Ophthalmol.*, **3**, 138–169 (1949).
- ² Auerbach, E., and Wald, G., *Science*, **120**, 401–405 (1954); *Am. J. Ophthalmol.*, **39**, suppl. 1, 24–40 (1955).
- ³ Das, S. R., *J. opt. Soc. Am.*, **54**, 541–546 (1964).
- ⁴ Watkins, R. D., *Vision Res.*, **9**, 1185–1196 (1969).
- ⁵ Mollon, J. D., and Polden, P. G., *Nature*, **258**, 421–422 (1975).
- ⁶ Wright, W. D., *Researches on Normal and Defective Colour Vision* (Kimpton, London, 1946).
- ⁷ Gouras, P., *J. Physiol., Lond.*, **199**, 533–547 (1968).
- ⁸ Du Croz, J. J., and Rushton, W. A. H., *J. Physiol., Lond.*, **183**, 481–496 (1966).
- ⁹ Norren, D. V., and Padmos, P., *Vision Res.*, **14**, 677–686 (1974).
- ¹⁰ Mollon, J. D., and Polden, P. G., *J. Physiol., Lond.*, **254**, 66P (1976).
- ¹¹ Baker, H. D., Doran, M. D., and Miller, K. E., *J. opt. Soc. Am.*, **49**, 1065–1070 (1959).
- ¹² Cornsweet, T., *Am. J. Psychol.*, **75**, 485–491 (1962).
- ¹³ Weale, R. A., *Nature*, **191**, 471–473 (1961).
- ¹⁴ Brindley, G. S., *J. Physiol., Lond.*, **122**, 332–350 (1953).

Restoration of sensitivity of cultured hepatoma cells to cyclic nucleotides shows permissive effect of dexamethasone

CULTURED rat hepatoma cell lines H-35, HTC and RLC respond to glucocorticoid hormones and have revealed details of how these steroids regulate the activity of tyrosine aminotransferase (TAT) that could not have been learned using intact liver^{1–4}. Unfortunately, the apparent lack of other regulatory mechanisms normally present in liver often limits the usefulness of these lines. For example, although cyclic AMP or its dibutyryl derivative reproducibly cause a several-fold induction of TAT in adult⁵ and foetal rat liver⁶ and in the H-35 (refs 7 and 8) and RLC⁹ hepatoma cell lines, these compounds have generally been found to be ineffective inducers of HTC cell tyrosine aminotransferase^{8–10}. Taken with the demonstration that HTC cells have marginally detectable levels of adenylate cyclase and cyclic AMP, this insensitivity to cyclic nucleotides led to the suggestion that glucocorticoid induction does not depend on cyclic AMP¹⁰. The following evidence suggests that the converse may not be true: TAT induction by dibutyryl cyclic AMP (db cyclic AMP) in adult rat liver is reduced by adrenalectomy¹¹; combinations of db cyclic AMP and steroids result in synergistic induction in rat liver and liver organ culture⁵; and cortisol increases the absolute degree of induction of TAT by db cyclic AMP in some responsive hepatoma cell lines^{8,12}. This putative inter-relationship could be clarified if a cell line, otherwise unresponsive to cyclic nucleotides, could be made to respond to these agents by adrenal steroid hormones. This report describes such an accomplishment and suggests that HTC cells are a model system for studies of the permissive effects of glucocorticoid hormones.

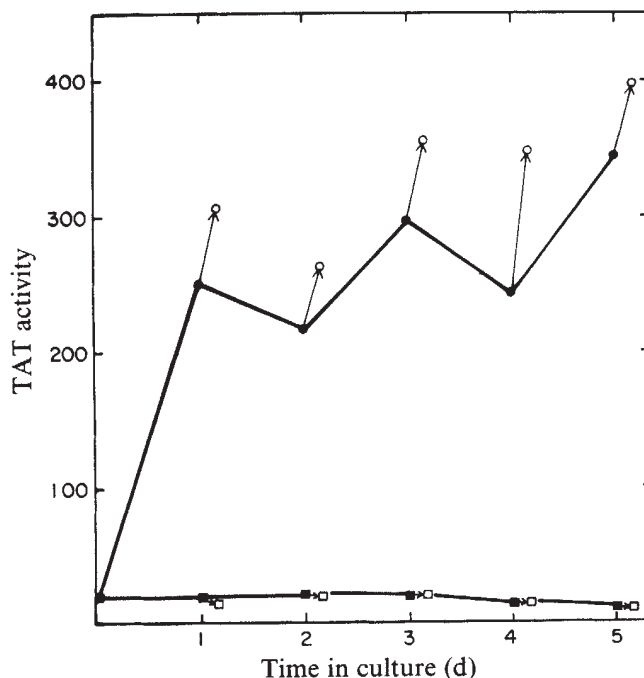
Addition of 1 mM db cyclic AMP to HTC cells grown in suspension for 1–5 d in modified Swim's S77 medium⁹ results in no increase in TAT activity over the basal value (Fig. 1). This lack of induction¹⁰ has repeatedly been demonstrated in our line of HTC cells and those of others^{8,9} and thus seems to represent a stable trait. Two groups have found a small (35–50%) increase in TAT activity when db cyclic AMP was added to monolayer cultures of HTC cells^{12,13}. If significant, this induction may reflect different culture conditions or variant strains, and resolution of this difference awaits further experimentation. Supplementation of the standard medium with 5 μ M dexamethasone results in 15- to 20-fold induction of HTC cell TAT, a process which has been shown to be attributable to an increased rate of synthesis of the enzyme³. Addition of 1 mM db cyclic AMP to these steroid-treated cells results in a further increase in TAT-specific activity (Fig. 1). This 'reinduction'—so-called because it is again possible to elicit a response associated with normal hepatocytes, and to differentiate it from superinduction⁴ and deinduction¹⁴ which define specific aspects of TAT regulation—is evident 24 h after addition of dexamethasone to the medium, persists for at least 5 d if hormone is kept in the

medium, and has been consistently demonstrated for the past 18 months. The extent of reinduction, greatest on day 4 of this experiment, represents an increase in activity of 100 mU per mg protein, and thus is equivalent to a tenfold induction over the basal value of 10 mU per mg protein. Reinduction requires specific nucleotides. Although *N*⁶-monobutyryl cyclic AMP is also effective, cyclic AMP, cyclic GMP, adenosine and sodium butyrate do not work (data not shown).

The kinetics of reinduction are shown in Fig. 2. TAT activity begins to increase 60–90 min after addition of db cyclic AMP and maximal levels are achieved between 4–6 h. This time course resembles that seen when db cyclic AMP is added to RLC cells, a cell line in which we have found TAT to be inducible in the absence of glucocorticoid hormones⁹.

To investigate whether reinduction of TAT in HTC cells requires protein synthesis, 0.1 mM cycloheximide was added 30 min before addition of db cyclic AMP. This concentration, which inhibits protein synthesis by 95% and prevents steroid induction if added before the hormone², also completely blocks the additional induction of TAT seen when 1 mM db cyclic AMP is added to HTC cells grown in medium supplemented with dexamethasone (Table 1). Thus continued protein synthesis is required for the expression of reinduction. Preliminary experiments using a highly specific anti-TAT antibody suggest that there is a parallel increase in TAT antigenic and catalytic activity with reinduction, and that there is a specific increase in the rate of synthesis of the enzyme. Reinduction thus seems

Fig. 1 Restoration of sensitivity of HTC cells to db cyclic AMP. HTC cells were grown in a modified Swim's S77 medium⁹ supplemented with 5% foetal calf and 5% calf sera heat inactivated at 56 °C for 30 min, or in this medium containing 5 μ M dexamethasone (gift of Merck, Sharpe and Dohme), and were diluted with the appropriate medium every other day to maintain cells in log growth. Aliquots of cells were removed from each culture daily and adjusted to a concentration of 500,000 cells ml⁻¹, then db cyclic AMP (Boehringer-Mannheim) was added to a final concentration of 1 mM. Four hours later TAT specific activity, equivalent to nmol *p*-hydroxyphenylpyruvate formed per mg protein per min, was determined⁹. Cells grown in absence of dexamethasone before (■) and after (→□) addition of db cyclic AMP; cells grown in the presence of dexamethasone before (●) and after (→○) addition of the nucleotide. Daily variation in TAT activity in cells grown in dexamethasone probably reflects induction from the serum in fresh medium²².



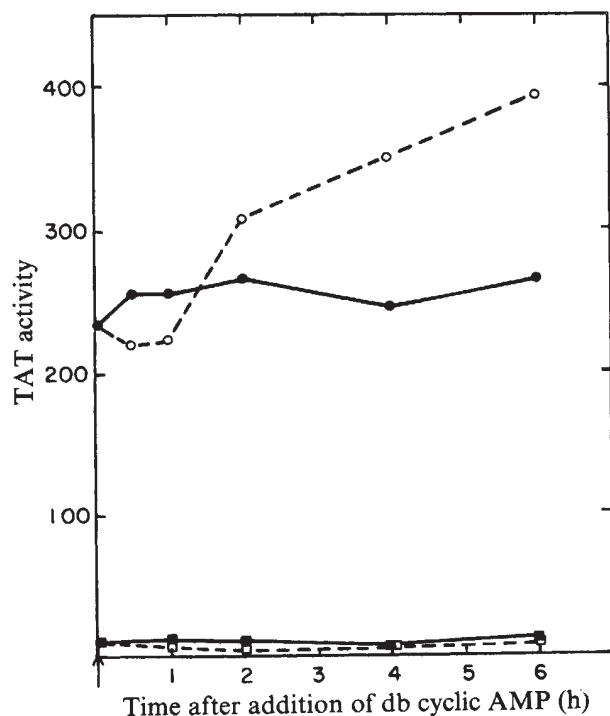


Fig. 2 Time curve of reinduction. HTC cells taken from cultures on day 4 of the experiment described in Fig. 1 were resuspended at a concentration of $500,000 \text{ cells ml}^{-1}$ in modified S77 medium $\pm$ dexamethasone. Dibutyl cyclic AMP (1 mM) was added to half of each culture and samples were taken for determination of TAT activity at times indicated on abscissa. Control cells without (□) or with (■) db cyclic AMP; dexamethasone-treated cells without (○) or with (●) nucleotide.

to involve mechanisms similar to the induction of TAT by steroid hormones³.

Shortly after glucocorticoid hormones were discovered, Ingle proposed that they have a general role in enabling maximal metabolic rates of processes essential to homeostasis¹³. Subsequently several permissive effects involving a variety of processes have been elucidated. Among these effects are those of adrenaline on gluconeogenesis¹⁴, glycogenolysis¹⁷ and lipolysis¹⁸, of mammary tumour virus production¹⁹, of glucagon on amino acid transport into isolated hepatocytes²⁰, and of growth factor stimulation of fibroblast proliferation²¹. The reinduction of TAT by db cyclic AMP in the strain of HTC cells used in this study is of interest because the steroid is not just enhancing a response (as in the other examples of TAT induction^{5,8,11,12}) but rather is absolutely required. Although little is known about the mechanism(s) of any permissive effect, a great deal is known about the regulation of TAT. A well defined cultured cell system is thus available for investigating this important action of glucocorticoid hormones.

The lack of regulatory mechanism in cultured cells is

Table 1 TAT activity (nmol per mg protein per min)

Additions	S77 medium	S77 + DEX medium
None	8.1	74.6
Dibutyl cyclic AMP	8.2	151.6
Dibutyl cyclic AMP + cycloheximide	7.9	87.2

Effect of inhibition of protein synthesis on reinduction. Cultures of HTC cells grown for 6 d in either S77 medium or S77 + dexamethasone, were each divided into three samples and resuspended at $500,000 \text{ cells ml}^{-1}$. Cycloheximide (0.1 mM, Sigma) was added to one sample from each culture; 30 min later 1 mM db cyclic AMP was added to each of these, and to a second sample. One sample from each original culture received nothing. Four hours after addition of the db cyclic AMP, TAT activity was determined in all samples. These results are representative of four similar experiments.

commonly ascribed to deletion or inactivation of genes during transformation or adaptation of cells to permanent growth in culture, but may also result from failure of the artificial milieu to support such processes. This demonstration of the restoration of a regulatory step by the addition of a hormone to the culture medium suggests that proper culture conditions may restore other apparently 'lost' functions in cultured hepatoma cells.

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DARYL K. GRANNER

Departments of Internal Medicine and Biochemistry,
University of Iowa and Veterans' Administration Hospitals,
Iowa City, Iowa 52240

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- Pitot, H. C., Peraino, C., Morse, P. A., Jr, and Potter, V. R., *Natn. Cancer Inst. Monogr.*, **13**, 229–245 (1964).
- Thompson, E. B., Tomkins, G. M., and Curran, J. F., *Proc. natn. Acad. Sci. U.S.A.*, **56**, 296–303 (1966).
- Granner, D. K., Thompson, E. B., and Tomkins, G. M., *J. biol. Chem.*, **245**, 1472–1478 (1970).
- Tomkins, G. M., *et al.*, *Science*, **166**, 1474–1480 (1969).
- Wicks, W. D., Kenney, F. T., and Lee, K. L., *J. biol. Chem.*, **244**, 6008–6013 (1969).
- Wicks, W. D., *J. biol. Chem.*, **246**, 217–223 (1971).
- Barnett, C. A., and Wicks, W. D., *J. biol. Chem.*, **246**, 7201–7206 (1971).
- Butcher, F. R., Becker, J. E., and Potter, V. R., *Expl Cell Res.*, **66**, 321–328 (1971).
- Granner, D. K., Sellers, L., Lee, A., Butters, C., and Kutina, L., *Archs Biochem. Biophys.*, **169**, 601–615 (1975).
- Granner, D. K., Chase, L., Aurbach, G., and Tomkins, G. M., *Science*, **162**, 1018–1020 (1968).
- Wicks, W. D., Barnett, C. A., and McKibbin, J. B., *Fedn Proc.*, **33**, 1105–1111 (1974).
- Stellwagen, R. H., *Biochem. biophys. Res. Commun.*, **47**, 1144–1150 (1972).
- Van Rijn, H., Bevers, M. M., Van Wijk, R., and Wicks, W. D., *J. Cell Biol.*, **60**, 181–191 (1974).
- Levinson, B., Tomkins, G. M., and Stellwagen, R. H., *J. biol. Chem.*, **246**, 6297–6302 (1971).
- Ingle, D. J., *J. Endocr.*, **8**, xxiii–xxvii (1952).
- Exton, J. H., *et al.*, *J. biol. Chem.*, **247**, 3579–3588 (1972).
- Shaffer, L. D., Chenoweth, M., and Dunn, A., *Biochim. biophys. Acta*, **192**, 292–303 (1969).
- Shafir, E., and Steinberg, D., *J. clin. Invest.*, **39**, 310–319 (1960).
- Dickson, C., Haslam, S., and Nandi, S., *Virology*, **62**, 242–252 (1974).
- Kletzien, R. F., Pariza, M. W., Becker, J. F., and Potter, V. R., *Nature*, **256**, 46–47 (1975).
- Armelin, H., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2702–2706 (1973).
- Gelehrter, T. D., and Tomkins, G. M., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 723–730 (1969).

Role of serum in protease-induced stimulation of 3T3 cell division past the monolayer stage

TREATMENT of a confluent, quiescent 3T3 mouse embryo fibroblast cell monolayer with low concentrations of various proteases is sufficient to induce a new round of cell division^{1,2}. It was suggested that modification of the 3T3 surface conformation from the non-agglutinable to the agglutinable state after protease treatment might have been responsible for the initiation of this round of cell division. More recently, however, Glynn *et al.*³ demonstrated that modification of the 3T3 cell surface from the agglutinable to the non-agglutinable state was not sufficient in itself to induce a new round of cell division. In the light of this and other unpublished work from our laboratory I have re-evaluated the role of various culture conditions in the induction of 3T3 cell division after a brief treatment with Pronase. I found that induction of cell division after treatment of a confluent 3T3 monolayer with protease depends both qualitatively and quantitatively on the serum in the growth medium and that this requirement varies from cell line to cell line. Furthermore, my results suggest that the modification in surface architecture detected as enhanced agglutinability after brief proteolysis is not sufficient to induce a new round of cell division.

As Table 1 shows, four of the 3T3 cell lines tested showed remarkably different serum requirements for growth to the monolayer stage, which we have somewhat arbitrarily put at 2.0×10^4 cells per cm^2 . For example, the cell line designated 3T3_a grows to the monolayer stage in Dulbecco's

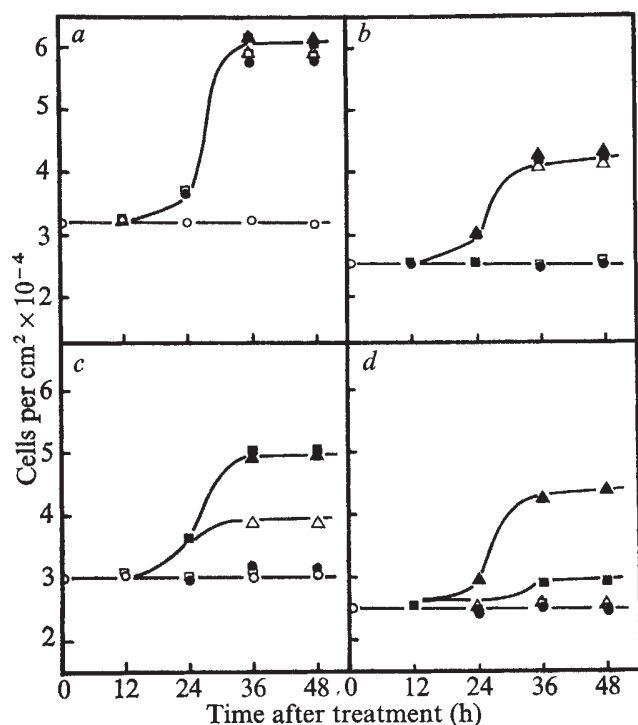


Fig. 1 Protease stimulation of different 3T3 cell lines in various concentrations of calf serum. The various 3T3 cell lines were plated at 2.0×10^3 cells per cm² and then grown to confluency in DME+10% calf serum. After 2 d at the monolayer stage the cells were washed once with sterile, phosphate-buffered 0.16 M NaCl (pH 7.2) (PBS). The cells were incubated for 5 min with 10 μ g of Pronase in 1.0 ml sterile PBS at room temperature. Cells were then washed with sterile PBS. DME plus the desired concentration of calf serum was then added back to the cells. Cells were counted every 12 h after stimulation. *a*, 3T3₃; *b*, 3T3₅; *c*, 3T3₇; *d*, 3T3₁₀. $\square$, DME+3% calf serum; $\triangle$, DME+5% calf serum; $\blacksquare$, DME+7% calf serum; $\blacktriangle$, DME+10% calf serum; $\bullet$, conditioned media; $\circ$, untreated monolayer. "Conditioned" medium is defined in the legend to Table 1. The untreated monolayer refers to cells which, after growth to the monolayer, were not treated with Pronase or fresh calf serum and which serve as a base line from which the percentage stimulation is determined.

modified Eagle's medium (DME) plus as little as 3% calf serum while the 3T3₁₀ cell line requires DME plus 10% calf serum to reach the monolayer. Cell lines 3T3₅ and 3T3₇ have intermediate serum requirements, growing to 2.0×10^4 cells per cm² in DME plus 5% or 7% calf serum, respectively. Although two of these cell lines (3T3₃ and 3T3₅) can grow to confluency in relatively low concentrations of calf serum, these lines do not significantly overgrow the monolayer when grown from low density in DME+10% calf serum.

I investigated the ability of DME plus various concentrations of calf serum to re-initiate cell division past the monolayer stage. As Table 2 shows, these cell lines vary considerably in terms of the concentration of calf serum necessary to stimulate a round of division past the monolayer. For example, 3T3₃ shows a 30% increase in cell number when DME+5% calf serum is added to the confluent cells whereas the other cell lines show no more than a 10% increase in cell number after a change of medium to DME+5% calf serum. In this same regard 3T3₃ cells show almost a 95% increase in cell number when DME+10% calf serum is added to the confluent cells, whereas 3T3₁₀ cells show only a 15% increase in cell number after a media change to DME+10% calf serum. 3T3₅ and 3T3₇ cells show responses to the various serum concentrations intermediate to 3T3₃ and 3T3₁₀. None of the cell lines shows more than a 15% increase in cell number when DME plus that concentration of serum necessary for growth to

confluency is added to cells which have been grown to the monolayer stage in DME+10% calf serum.

To determine whether the differences in serum concentration required for subconfluent growth influenced the ability of the cells to overgrow the monolayer after protease treatment, each cell line was grown to confluency in DME+10% calf serum and then pulsed with Pronase as described in the legend to Fig. 1. As can be seen, there was significant induction of cell division after protease treatment only at a concentration of calf serum equal to or above that required for growth to the monolayer stage. After stimulation of each cell line in medium containing the minimal concentration of fresh calf serum sufficient to maintain cell division at a subconfluent density, a 65–75% increase in cell number was observed within 30 h of the initial stimulation. Only the 3T3₃ cell line showed a greater than 75% increase in cell number after treatment with Pronase.

To evaluate properly the effect of protease on the induction of cell division it is necessary to compare the stimulation of cell division observed before and after addition of fresh calf serum. Comparison of Fig. 1 and Table 2 shows that protease treatment lowered the concentration of fresh calf serum necessary for significant induction of cell division past the monolayer. For example, whereas 3T3₃+DME containing 3% calf serum showed only a 10% induction of cell division (Table 2), 3T3₃ cells treated with protease before addition of DME+3% serum showed a 90% increase in cell number. Similar results have been obtained with the other three cell lines (compare Fig. 1 and Table 2).

Fig. 2 Effect of ISI and Gibco calf serum on subconfluent cell growth and protease-stimulated overgrowth. 3T3₁₀ cells were plated at 2.5×10^3 cells per cm² in DME+10% ISI calf serum or 10% Gibco calf serum. Cell counts were taken daily during the next 6 d. Inset: 3T3₁₀ cells grown to confluency in DME+10% ISI calf serum or 10% Gibco calf serum were treated with 10 μ g of Pronase per ml PBS and then DME+10% ISI or 10% Gibco calf serum was added back to the cells at the monolayer. Cell counts were performed every 12 h for the next 72 h. $\bullet$, DME containing 10% Gibco calf serum; $\blacksquare$, DME containing 10% ISI calf serum; $\circ$, Pronase 10 μ g ml⁻¹ + DME containing 10% Gibco calf serum; $\square$, Pronase 10 μ g ml⁻¹ + DME containing 10% ISI calf serum. Addition of fresh 10% Gibco calf serum to cells at the monolayer without previous protease treatment produced an increase in cell number of approximately 15%. The addition of fresh 10% ISI serum to cells at the monolayer without previous protease treatment produced less than a 10% increase in cell number.

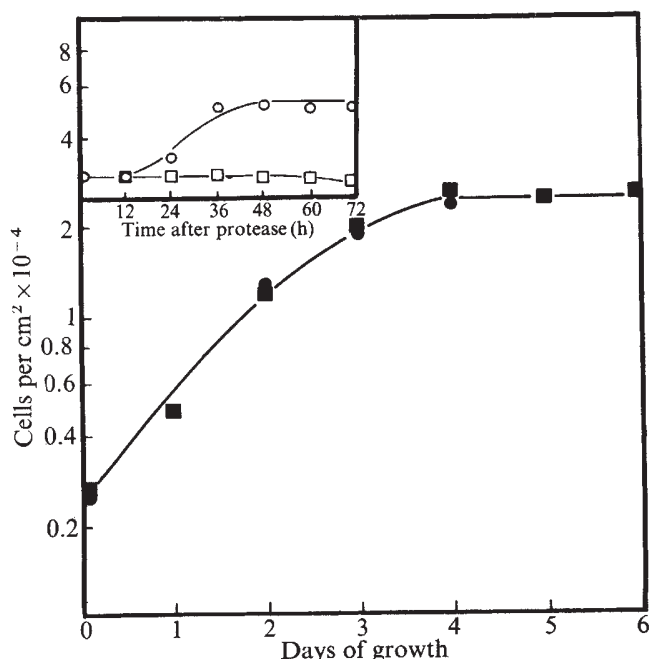


Table 1 Serum requirements of individual cell lines

Cell line	Final cell density cells per cm ² × 10 ⁻⁴					Conditioned medium
	DME+3% calf serum	DME+5% calf serum	DME+7% calf serum	DME+10% calf serum		
3T3 ₃	2.2	2.5	3.0	3.2		2.5
3T3 ₅	1.5	2.0	2.4	2.6		1.75
3T3 ₇	0.35	1.0	2.5	3.0		0.75
3T3 ₁₀	0.30	0.675	1.1	2.5		0.30

Saturation density was determined by plating cells at approximately 2.0×10^3 per cm² in DME plus the given concentration of calf serum and counting cells daily for the next 7 d (ref. 2). A complete monolayer is formed with these cells at approximately 2.0×10^4 cells per cm². Cell size remained approximately the same for all cell lines tested. Cell lines originally derived from an NIH Swiss mouse embryo fibroblast (3T3) cell line were used throughout. The original line was obtained as clone 41 from Dr G. J. Todaro. Random stocks of this original clone had been stored in liquid nitrogen at different intervals during the past 5 yr. Four of these cell stocks were used. All cell lines were maintained at 37.5 °C in a moist incubator in which the CO₂ tension was held at 5%. Dulbecco's modified Eagle's medium (DME) plus 10% calf serum and 1% penicillin-streptomycin was used as growth medium for all stock cultures. The cells were demonstrated to be free of contamination with pleuro-pneumonia-like organisms (PPLO) at the time of the experiments, by both autoradiography⁶ and mycoplasma broth media⁷. No cell line was maintained in culture for more than 20 passages. Frozen stocks were removed from liquid N₂ at intervals during the course of the experiments. "Conditioned" medium was produced by plating each cell line at 2.0×10^3 cells per cm² in DME+10% calf serum and allowing the cells to grow in this medium for 5 d at which time the medium was removed and filtered. In this regard it must be noted that the term "conditioned" medium refers to medium taken from specific cell lines. Thus the "conditioned" medium which is added back to 3T3₃ cells is media which have been depleted by 3T3₃ cells while "conditioned" media which are added to 3T3₁₀ cells have been depleted by 3T3₁₀ cells. Since these cell lines have different serum requirements for growth it might be expected that the "conditioned" media produced by the two cell types might also vary.

Significantly, comparison of Table 2 and Fig. 1 demonstrates that 3T3₃ is the only cell line which, after growth to confluency, can be stimulated to divide in the presence of "conditioned" medium (see legend to Table 1 for definition of "conditioned" medium).

To determine whether the ability of serum to support growth past the monolayer varied with different lots of calf serum, Pronase stimulation of 3T3₁₀ cells was studied with three different sera. Figure 2 shows that subconfluent

It has been suggested that the surface modification detected as enhanced agglutinability with plant lectins, which results from mild protease treatment of a confluent monolayer, might be necessary for induction of cell division past the monolayer stage¹.

The surface change detected as enhanced agglutinability is not sufficient for induction of cell division past the monolayer stage. Although 3T3₁₀ cells treated with 10 µg of Pronase per ml of sterile phosphate-buffered saline show

Table 2 Initiation of cell division past the monolayer by serum

Cell line	Final cell density per cm ² × 10 ⁻⁴	Cell density (per cm ² × 10 ⁻⁴) after addition of DME at the indicated concentration of fresh calf serum to confluent cells				Conditioned medium
		3%	5%	7%	10%	
3T3 ₃	3.2	3.5	4.16	5.6	6.2	3.7
3T3 ₅	2.6	2.6	2.9	3.12	4.55	2.8
3T3 ₇	3.0	3.2	3.3	3.5	4.1	3.0
3T3 ₁₀	2.6	2.6	2.4	2.7	2.9	2.4

Each cell line was grown to confluency in DME+10% calf serum. The cells were left at confluency for 48 h and then the medium was changed to DME + the desired concentration of calf serum. Cell counts were performed every 12 h. In this table only the final cell density reached 48 h after the change of media is reported. "Conditioned" medium is defined in the legend to Table 1.

growth of the 3T3₁₀ cells is identical in calf serum obtained from Gibco (Lot No. C345417) and ISI (Lot No. 716C312). But as Fig. 2 shows, this lot of ISI sera did not support protease-induced cell division past the monolayer stage. This suggests that the serum components necessary for protease induction of cell division past the monolayer are either present in reduced amounts or missing completely from this lot of ISI sera. Calf serum obtained from Flow Laboratories was similar in its growth promoting characteristics to that of the Gibco calf serum used throughout these experiments.

enhanced agglutinability with wheat germ agglutinin and concanavalin A immediately after treatment (Table 3), these cells will complete a round of cell division only when exposed to DME plus fresh 10% calf serum (Fig. 1). Thus, as Glynn *et al.* suggested³, modification of the surface architecture from the non-agglutinable to the agglutinable state is not sufficient in itself to reinitiate the cell cycle in 3T3 cells at high density.

The work presented here suggests that the efficiency of protease-induced reinitiation of cell division varies from cell clone to cell clone, even within the 3T3 cell line. The

Table 3 Effect of protease on 3T3₁₀ cell growth and agglutination

Cell line	Final cell density (cells per cm ² × 10 ⁻⁴)		% Agglutination			
	Control	Plus Pronase	25 µg ml ⁻¹ con A	25 µg ml ⁻¹ WGA	25 µg ml ⁻¹ con A	25 µg ml ⁻¹ WGA
3T3 ₁₀	3.0	4.8	10%	10%	90%	95%

All agglutinations were performed as before². DME+10% calf serum was added to the 3T3₁₀ cells after Pronase treatment. In the growth experiments "control" 3T3₁₀ cells were treated with DME+10% calf serum without previous Pronase treatment. With regards agglutination, control cells refer to cells before Pronase treatment. All protease treatments were performed with 10 µg Pronase per ml of sterile PBS for 5 min at 22 °C. Concanavalin A was prepared according to the techniques of Agrawal and Goldstein⁴, while wheat germ agglutinin (WGA) was prepared according to Nagata and Burger⁵.

relative efficiency of "protease-induced overgrowth" of the monolayer seems to correlate with the serum requirement which each cell clone expresses for sub-confluent growth. Thus in our work only those 3T3 cell lines which can grow to the monolayer in 3% calf serum seem able to respond to protease-induced reinitiation of the cell cycle without requiring the further addition of fresh serum to the growth medium.

In addition to this quantitative serum requirement there seems to be a qualitative requirement for a serum component(s) which is apparently not required for subconfluent growth but which is essential for growth past the monolayer. This conclusion stems from the observation that some calf sera which I tested, although able to support subconfluent growth, were unable to support growth of Pronase-treated cells past the monolayer.

Continuing work in our laboratory suggests that 3T3 cells may be peculiarly susceptible to induction of cell division after limited proteolysis. Attempts to induce other murine and rodent cell lines to reinitiate cell division after Pronase treatment have proved unsuccessful, even in media conditions which the work reported here suggests should be adequate for overgrowth.

Thus a working hypothesis is that limited protease digestion of the 3T3 cell membrane may act to increase the efficiency of utilisation of serum by a cell at the monolayer, thereby reinitiating cell division past the monolayer. This reinterpretation of earlier work is in substantial agreement with Holley's suggestion⁸ that modification of the cell membrane may serve to increase the transport of serum nutrients or growth factors into the cell, and in this way lead to a reinitiation of the cell cycle. Reference 9 has a more extended discussion of the role of protease in cell growth.

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KENNETH D. NOONAN

Department of Biochemistry,
J. Hillis Miller Health Center,
University of Florida,
Gainesville, Florida 32610

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- ¹ Burger, M. M., *Nature*, **227**, 170-171 (1970).
- ² Noonan, K. D., and Burger, M. M., *Exp. Cell Res.*, **80**, 405-414 (1973).
- ³ Glynn, R. D., Thrash, C. R., and Cunningham, D. D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2676-2679 (1973).
- ⁴ Agrawal, B. B. L., and Goldstein, A. J., *Biochem. J.*, **96**, 23c-25c (1965).
- ⁵ Nagata, Y., and Burger, M. M., *J. Biol. Chem.*, **247**, 2248-2250 (1972).
- ⁶ Culp, L. A., and Black, P. H., *Biochemistry*, **11**, 2161-2172 (1972).
- ⁷ Hayflick, L., and Stanbridge, E., *Ann. N.Y. Acad. Sci.*, **143**, 608-621 (1967).
- ⁸ Holley, R. W., *Proc. natn. Acad. Sci., U.S.A.*, **69**, 2840-2841 (1972).
- ⁹ *Proteases and Biological Control*, (edit. by Reich, E., Rifkin, D., and Shaw, E.) (Cold Spring Harbor Laboratory, New York, 1975).

Specificity and reversibility of interferon ganglioside interaction

RECENT studies have indicated that antiviral action of interferon is triggered by interaction with the cell membrane: Sepharose-bound mouse interferon (IF-Sepharose) retains its antiviral activity, and direct contact with the target cells is necessary to produce the antiviral effect^{1,2}. Cells stimulated by double-stranded polyribonucleic-polyribocytidylic acid to produce interferon are not protected from viral infection in the presence of interferon antiserum, indicating that interferon acts only after it has left the cells in which it is produced³. The same conclusion has been drawn from experiments with ouabain which inhibits the action, but not the production of primate interferon: cells stimulated to produce interferon fail to become antiviral in the presence of ouabain⁴.

We have shown previously that preincubation of mouse

L cells with *Phaseolus vulgaris* phytohaemagglutinin (PHA) blocks interferon action, suggesting the involvement of PHA-binding sites on the cell surface in cell membrane-interferon interaction⁵. We further demonstrated that Sepharose-bound mouse interferon is inhibited after preincubation with gangliosides, that soluble interferon binds to Sepharose-bound gangliosides, and that binding to gangliosides is inhibited by PHA⁶. Since gangliosides are cell membrane constituents whose carbohydrate portion faces the outside of the cell, we have investigated whether interferon-ganglioside interaction involves binding to the carbohydrate moiety of the gangliosides, thus supporting our hypothesis that interferon can interact with gangliosides on the outside of the cell membrane *in vivo*.

As Table 1 shows, when mouse IF-Sepharose was incubated with various glycolipids at comparable concentrations, pronounced inhibition of antiviral activity was observed with G_{M2}, in accord with previous results⁶. Very little inhibition by G_{M3} was seen in the conditions used, suggesting that the terminal *N*-acetyl-galactosaminyl residue is an important constituent of the inhibitory molecule (see legend to Table 1). The same is true for terminal sialic acid as shown by the lack of inhibition obtained with ganglioTri-Cer. Both globoTri-Cer and globoTet-Cer (globoside) likewise were not inhibitory. They differ from G_{M2} by the absence of sialic acid and by the fact that the galactosyl residue of lactosyl ceramide is substituted by an α -1,4-linked galactosyl residue (globoTri-Cer) which in globoTet-Cer is further substituted by β -1,3-linked *N*-acetyl-galactosamine. GloboTri-Cer resulted in a slightly turbid solution in water, whereas ganglioTri-Cer yielded a turbid suspension, yet finely dispersed. Therefore in further experiments IF-Sepharose was preincubated with ethanolic solutions of the glycolipids. IF-Sepharose was first washed with absolute ethanol, which had little effect on its antiviral activity, then incubated with glycolipid solutions in ethanol followed by washing with water as described in the legend to Table 1. In these conditions only globoTet-Cer remained largely insoluble. The data corroborate those obtained with aqueous solutions, though G_{M3} showed slight inhibition of antiviral activity. It seems that in ethanol, lower concentrations of G_{M2} are required for complete inhibition, presumably because it forms micelles when dissolved in water. Since modification of the carbohydrate portion of G_{M2} results in reduced inhibition or no inhibition, it seems that inhibitory ganglioside molecules bind through their carbohydrate portion to the interferon molecule and that optimal binding and inhibition require a lactosyl residue with galactose substituted by both a 2,3-linked sialic acid and a β -1,4-linked *N*-acetyl-galactosamine.

There is further support that ganglioside binding to interferon is not due to interaction of its lipid portion with hydrophobic sites on the interferon molecule. It was not possible to restore antiviral activity by washing IF-Sepharose, completely inhibited after preincubation with bovine brain gangliosides⁶, with 50% ethylene glycol. Such treatment reverses hydrophobic binding of human interferon to Sepharose columns containing covalently linked albumin¹², or to CH-Sepharose¹³.

If inhibition of antiviral activity by gangliosides is due to specific interaction of interferon with carbohydrate constituents on the ganglioside molecule, it should be possible to reverse inhibition using saccharides resembling part or all of the carbohydrate structure of G_{M2}. As Table 2 shows, when ganglioside-inhibited IF-Sepharose beads were washed with sialyl-lactose at pH 2, almost all antiviral activity reappeared. Washing with lactose or sialic acid alone had little effect. But when both sialic acid and lactose were present, antiviral activity was partially restored. Thus interferon seems to act like a lectin that is specific for at least the sialyl-lactose portion on the ganglioside molecule. At pH 7.0 and comparable concentrations neither sialyl-

Table 1 Inhibition of antiviral activity of IF-Sephacrose by glycolipids

IF-Sephacrose preincubated with	Virus yield (% of control) after treatment of L cells with IF-Sephacrose preincubated with glycolipids in:	
	Water	Ethanol
Nothing	0.7	2.5
G _{M2}	51*	100
G _{M3}	1	16
ganglioTri-Cer	1	5
globoTri-Cer	0.8	4.7
globoTet-Cer	1.2	5

IF-Sephacrose prepared as before^{1,2} was treated with glycolipids by incubating 5×10^4 beads with 0.3 ml of a glycolipid solution in either water (3 mg ml^{-1}) or absolute ethanol (1 mg ml^{-1}). Beads preincubated with ethanolic solutions were washed first with absolute ethanol (twice, 2 ml). Incubation was for 75 min at 37°C . The beads were then washed twice with 2 ml water and suspended in 1 ml Eagle's minimal essential medium plus Hanks salts without serum (MEM). G_{M2} was obtained from human brain⁷, G_{M3} from bovine spleen⁷, ganglioTri-Cer from guinea pig erythrocytes⁸, globoTri-Cer from rabbit erythrocytes⁹, and globoTet-Cer from pig heart⁹. All glycolipids were essentially pure compounds according to thin layer chromatography and gas-liquid chromatography-mass spectrometry^{10,11}. Suspensions of glycolipids not immediately soluble were heated to 50°C followed by two to four ultrasonic shocks of 2-3 s in a Heat Systems Sonifier (Model W185) at 50-W output. Mouse L cells were cultivated at 37°C in 35-mm plastic dishes (10^6 cells per dish) in MEM, containing 10% calf serum. After removal of medium, cells were incubated for 6 h at 37°C with 1-ml suspensions of IF-Sephacrose at a ratio of one bead per 20 cells. After removal of beads, cells were challenged with encephalomyocarditis virus (EMC) at a multiplicity of infection of 1. Virus yield was determined after 16 h of incubation at 37°C by counting plaque-forming units (PFU). The control virus yield per plate in the absence of interferon was 6×10^7 PFU for the experiment with aqueous solutions and 1.6×10^8 PFU for that with ethanolic solutions of glycolipids. We reported previously inhibition of mouse IF-Sephacrose by a preparation of G_{M3} containing both the acetyl and the glycolyl neuraminic acid derivatives⁸. Since that experiment was not carried out simultaneously with those testing the effects of the other individual gangliosides and since a different preparation of IF-Sephacrose was used, the observed degree of inhibition by G_{M3} in our earlier report is not directly comparable with that of the other gangliosides and to the results reported here. We found subsequently that different preparations of IF-Sephacrose are inhibited to a different extent by the same quantity of a given ganglioside in the preincubation mixture. But in all IF-Sephacrose preparations tested the relative order of effectiveness of the gangliosides as inhibitors remained the same: $G_{M2} \geq G_{T1} > G_{M1} \geq G_{D1a} > G_{M3}$. These variations might be due to different amounts of contaminating proteins or glycoproteins bound to the Sephacrose particles, as the interferon preparations used for coupling to Sephacrose were not purified extensively^{1,2}. When the same IF-Sephacrose preparation was used, the degree of inhibition observed by a given amount of a ganglioside was reproducible in different experiments. Gangliosides are designated according to Svennerholm⁷: G_{M3}, 3-O-(N-acetyl) neuraminyl-4-O-β-galactosyl-O-glucosyl-ceramide; G_{M2}, 4-O-β-N-acetyl-galactosaminyl (3-O-(N-acetyl)neuraminyl)-4-O-β-galactosyl-O-glucosyl-ceramide; G_{M1}, 3-O-β-galactosyl-4-O-β-N-acetyl-galactosaminyl (3-O-(N-acetyl)neuraminyl)-4-O-β-galactosyl-O-glucosyl-ceramide; G_{D1a}, 3-O-(N-acetyl)neuraminyl-3-O-β-galactosyl-4-O-β-N-acetyl-galactosaminyl (3-O-(N-acetyl)neuraminyl)-4-O-β-galactosyl-O-glucosyl-ceramide; G_{T1}, 3-O-(N-acetyl)neuraminyl-3-O-β-galactosyl-4-O-β-N-acetyl-galactosaminyl (8-O-(N-acetyl)neuraminyl-3-O-(N-acetyl)neuraminyl)-4-O-β-galactosyl-O-glucosyl-ceramide. Other abbreviations used are: globoTri-Cer, 4-O-α-galactosyl-4-O-β-galactosyl-O-glucosyl-ceramide; globoTet-Cer, 3-O-β-N-acetyl-galactosaminyl-4-O-α-galactosyl-4-O-β-galactosyl-O-glucosyl-ceramide; ganglioTri-Cer, 4-O-β-N-acetyl-galactosaminyl-4-O-β-galactosyl-O-glucosyl-ceramide; sialyl-lactose, 3-O-(N-acetyl)neuraminyl-4-O-β-galactosyl-O-glucose. All sugars are of the D configuration. *At 2 mg ml^{-1} in the preincubation mixture.

lactose nor the mixture of lactose and sialic acid restored antiviral activity of ganglioside-inhibited IF-Sephacrose to any great extent, suggesting that the negative charge on the sialyl residues at neutral pH impedes replacement of the ganglioside molecules from interferon. It should be pointed out that in the determinations of antiviral activity only differences greater than twofold were considered significant.

We could not assess whether the complete tetrasac-

charide unit of G_{M2} is superior to sialyl-lactose in displacing inhibitory gangliosides from IF-Sephacrose, since this tetrasaccharide was not available. In displacement experiments in which suboptimal concentrations of sialyl-lactose were used during washing of ganglioside-inhibited IF-Sephacrose beads in otherwise identical conditions, N-acetyl-galactosamine did not enhance the effect of sialyl-lactose. Thus there was no difference in the virus yield after treatment of L cells with ganglioside-inhibited IF-Sephacrose subsequently washed at pH 2.0 or pH 7.0 with 0.02 M sialyl-lactose, compared with experiments in which a solution containing 0.02 M sialyl-lactose plus 0.5 M N-acetyl-galactosamine was used for washing. Similarly, when 0.5 M N-acetyl-galactosamine was included with 0.1 M lactose and 0.1 M sialic acid in the solution used to remove bound gangliosides from IF-Sephacrose, the effect observed with the mixture of sialic acid and lactose alone was not enhanced.

Since most glycoproteins contain terminal sialic acid often bound to penultimate galactosyl units, we surveyed some of these glycoproteins for possible inhibitory action on the antiviral activity of interferon. In these experiments 5×10^4 IF-Sephacrose beads were preincubated with 0.3 ml glycoprotein solution in water for 1 h at 37°C followed by washing with phosphate-buffered saline (PBS) (twice 2 ml). None of the following materials (20 mg ml^{-1}) had any effect on antiviral activity: Fetusin (Sigma), bovine submaxillary mucin (Sigma), rabbit α- and γ-globulins (Nutritional Biochemicals), human chorionic gonadotropin (Ayerst). Thus inhibition of IF-Sephacrose not only requires the presence of sialyl or sialyl-galactosyl residues on a glycoconjugate, but seems to be more specific.

The fact that shortening of the saccharide portion of G_{M2} can partially or completely prevent inhibition of interferon, supports our conclusion that interferon interacts with the carbohydrate moiety of this molecule. Substitution of the G_{M2} tetrasaccharide unit by β-1,3-linked galactose as in G_{M1} and higher gangliosides, has little influence on inhibitory potency⁸. Reversal of ganglioside inhibition by sialyl-lactose indicates that at least part of the binding site on the inter-

Table 2 Effect of saccharide solutions on ganglioside-inhibited IF-Sephacrose

Ganglioside-pretreated IF-Sephacrose washed with:	Virus yield (% of control) after incubation of L cells with ganglioside-pretreated IF-Sephacrose washed subsequently at	
	pH 2	pH 7
Buffer	54	72
0.1 M sialic acid	26	25
0.1 M lactose	35	50
0.1 M lactose plus 0.1 M sialic acid	8	30
0.7 M lactose plus 0.7 M sialic acid	11	ND
0.1 M sialyl-lactose	1.8	26
IF-Sephacrose not preincubated with gangliosides	0.3	0.3

IF-Sephacrose (5×10^4 beads) was incubated with 0.3 ml of a solution of mixed gangliosides from bovine brain (Sigma, type III, 2 mg ml^{-1}) for 75 min at 37°C . The beads were washed twice with 2 ml of water to remove unbound gangliosides. They were then suspended in 0.4 ml of the indicated saccharide solutions either in 0.2 M glycine-HCl buffer at pH 2.0 or in 0.3 M sodium phosphate buffer, pH 7.0. The pH was readjusted where necessary. After incubation at 37°C for 30 min with occasional stirring the supernatant solution was removed, and the beads were washed twice more with 2 ml of water and finally suspended in 1 ml of MEM. The major constituents present in the mixed ganglioside preparation used were G_{M1} and G_{D1a}, as revealed by thin-layer chromatography in chloroform-methanol-2.5 N ammonia (60:40:9; v/v). The sialyl-lactose used was from bovine colostrum (Sigma) and contained approximately 85% of the 2,3-linked and approximately 15% of the 2,6-linked N-acetylneuraminyl derivatives. Antiviral activity was determined as described in the legend to Table 1. The control virus yield per plate was 10^8 PFU. ND, not determined.

feron molecule is directed towards this trisaccharide unit. The fact that neither sialic acid nor lactose alone can reverse inhibition of IF-Sepharose by gangliosides, whereas a mixture of both is effective to some extent, supports our conclusion that the binding site for interferon includes both sugar residues. This is analogous to the observed inhibition of PHA-induced red cell agglutination by an erythrocyte glycopeptide that contains the PHA receptor site: although removal of galactose from this glycopeptide abolishes its agglutination-inhibitory activity, neither galactose alone nor β -galactosyl-*N*-acetyl-glucosaminyl-containing oligosaccharides are inhibitors of PHA-induced haemagglutination. Thus, although galactosyl residues are essential for PHA binding, the complete binding site seems to include further saccharide units¹⁴.

The relatively poor inhibition observed with G_{M3} suggests that optimal binding of interferon to gangliosides includes interaction with the β -1,4-linked *N*-acetyl-galactosaminyl residue as well. This hypothesis could be substantiated further by comparing the relative efficiencies of removing inhibitory gangliosides from IF-Sepharose using either sialyl-lactose or the tetrasaccharide derived from G_{M2}. Thus with the latter, lower concentrations should be sufficient to restore completely antiviral activity of ganglioside-inhibited IF-Sepharose. It is not obvious why sialyl-lactose reverses ganglioside inhibition at pH 2.0 but not at pH 7.0. Possibly the negative charges on the ganglioside molecules at pH 7.0 repel the negatively charged sialyl-lactose residues sufficiently to prevent successful competition of the trisaccharide with the bound gangliosides. This phenomenon would not occur at pH 2.0, where sialyl residues are uncharged.

Cells transformed by RNA and DNA viruses often have altered ganglioside patterns^{15,16}. For example, transformation of mouse cells by murine sarcoma virus (Moloney strain) yields decreased levels of G_{M2} and higher gangliosides in the cell membrane¹⁷. Virus transformation often results in decreased sensitivity to the antiviral effect of interferon¹⁸. Involvement of higher gangliosides in the cell membrane receptor sites for interferon could explain the poor antiviral response of some transformed cells. Preincubation of SV40-transformed mouse cells with gangliosides increases their sensitivity towards the antiviral effect of interferon, in accord with such a hypothesis¹⁹.

Binding of interferon to Sepharose-bound gangliosides⁶ and elution with the appropriate saccharide solutions suggests a way to purify interferon by affinity chromatography. Such studies are in progress.

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FRANCOISE BESANCON
HELMUT ANKEL

Department of Biochemistry,
Medical College of Wisconsin,
Milwaukee, Wisconsin 53233

SUBHASH BASU

Department of Chemistry,
Biochemistry and Biophysics Program,
University of Notre Dame,
Notre Dame, Indiana 46556

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¹ Ankel, H., Chany, C., Galliot, B., Chevalier, M. J., and Robert, M., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2360-2363 (1973).

- ² Chany, C., Ankel, H., Galliot, B., Chevalier, M. J., and Grégoire, A., *Proc. Soc. exp. Biol. Med.*, **147**, 293-299 (1974).
- ³ Vengris, V. E., Stollar, B. D., and Pitha, P. M., *Virology*, **65**, 410-417 (1975).
- ⁴ Lebon, P., Moreau, M. C., Cohen, L., and Chany, C., *Proc. Soc. exp. Biol. Med.*, **149**, 108-112 (1975).
- ⁵ Besançon, F., and Ankel, H., *Nature*, **250**, 782-785 (1974).
- ⁶ Besançon, F., and Ankel, H., *Nature*, **252**, 478-480 (1974).
- ⁷ Svennerholm, L., *J. Neurochem.*, **10**, 613-623 (1963).
- ⁸ Basu, M., Chien, J.-L., and Basu, S., *Biochem. biophys. Res. Commun.*, **60**, 1097-1104 (1974).
- ⁹ Chien, J.-L., Williams, T., and Basu, S., *J. biol. Chem.*, **248**, 1778-1785 (1973).
- ¹⁰ Björndal, H., Hellqvist, C. G., Lindberg, B., and Svensson, S., *Angew. Chem. Int. Engl.*, **9**, 610-619 (1970).
- ¹¹ Sung, S. J., Esselman, W. J., and Sweeley, C. C., *J. biol. Chem.*, **248**, 6528-6533 (1973).
- ¹² Huang, J. W., et al., *J. biol. Chem.*, **249**, 4665-4667 (1974).
- ¹³ Davey, M. W., Huang, J. W., Sulkowski, E., and Carter, W. A., *J. biol. Chem.*, **250**, 348-349 (1975).
- ¹⁴ Kornfeld, R., and Kornfeld, S., *J. biol. Chem.*, **245**, 2536-2545 (1970).
- ¹⁵ Brady, R. O., and Fishman, P. H., *Biochim. biophys. Acta*, **355**, 121-148 (1974).
- ¹⁶ Hakomori, S., *J. Adv. Cancer Res.*, **18**, 265-315 (1973).
- ¹⁷ Mora, P. T., Fishman, P. H., Bassin, R. H., Brady, R. O., and McFarland, V. W., *Nature new biol.*, **245**, 226-229 (1973).
- ¹⁸ Brailovsky, C. A., Berman, L. D., and Chany, C., *Int. J. Cancer*, **4**, 194-203 (1969).
- ¹⁹ Pitha, P. M., Vengris, V. E., and Reynolds, F. H., *Proc. ICN-UCLA Winter Conf. molec. cell. Biol.*, Squaw Valley (in the press).

Thrombin-sensitive surface protein of cultured chick embryo cells

THROMBIN, a serine protease with great specificity, is a potent mitogen for resting chick embryo fibroblasts in culture¹⁻³. So far only a limited number of peptide bonds in a few native proteins have been found to be sensitive to proteolysis by thrombin, namely four Arg-Gly bonds in fibrinogen⁴ and a few arginyl and lysyl bonds in actin⁵. It is likely therefore that the number of fibroblast surface proteins susceptible to this protease will also be limited, whereas with trypsin many surface proteins are attacked. Thus thrombin may be an excellent probe for studies of the role of changes in cell surface composition during mitogenesis or cell-cell interaction. A large, external transformation sensitive (LETS) protein⁶⁻¹⁰, postulated^{10,11} to be involved in cellular growth control, is insensitive to thrombin⁷. This observation led to the conclusion that the removal of LETS is not a necessary condition for cell growth when a protease is used to stimulate growth.

Using a lactoperoxidase-catalysed ¹²⁵I-iodine labelling technique, we were unable previously to detect decisively any thrombin-sensitive chick fibroblast surface protein. Yet since thrombin has a potent effect on chick embryo fibroblasts, it seemed likely that some surface protein is sensitive to its proteolytic activity. Identification of such a protein may be important not only for the understanding of cell surface composition, but also for the elucidation of the mechanism of protease-induced mitogenesis.

We have found that when ¹³¹I-iodine is substituted for ¹²⁵I-iodine in lactoperoxidase-catalysed iodination, the method greatly increases the number of surface proteins detectable by gel electrophoresis and autoradiography. This observation led to the finding reported here that there is a thrombin-sensitive protein with an apparent molecular weight of 205,000 on the surface of chick cells.

Until recently, the usual procedure for iodination of proteins has involved ¹²⁵I-iodide, mainly because of its more convenient long half life (60 d). When autoradiograms of slab gels of solubilised, iodinated cells are made, however, ¹²⁵I-iodide yields poor resolution because of its high energy gamma emission. A β -emitting iodine isotope would overcome this difficulty, since β radiation is captured more efficiently by the photographic emulsion than is γ radiation; the scattering effect is greatly reduced and resolution much improved. The combination of the use of ¹³¹I-iodide instead of ¹²⁵I-iodide and a gradient polyacrylamide gel is recommended. The improvement in analytical power offsets the inconvenience of a shorter half life (8 d).

As Fig. 1 shows, use of ¹³¹I-iodide and electrophoresis on sodium dodecyl sulphate (SDS)-polyacrylamide gradient slab gels makes possible detection of a large number

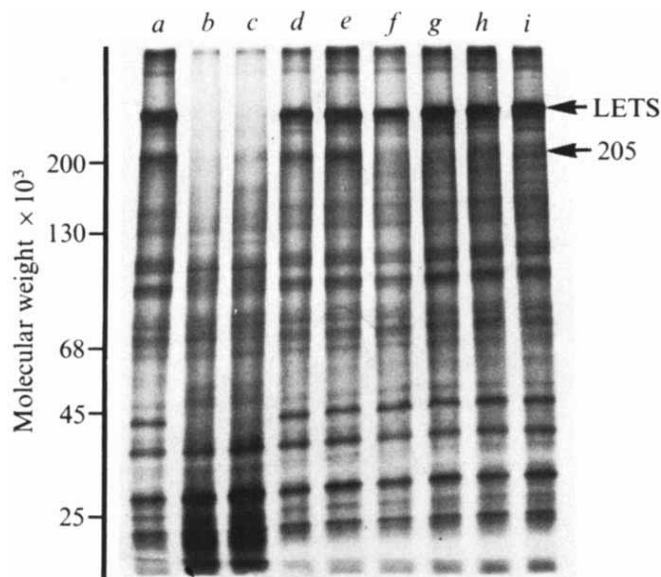


Fig. 1 Autoradiograms of SDS-polyacrylamide gradient gels of proteins labelled by lactoperoxidase-catalysed iodination of chick fibroblasts after the following treatment at 37 °C in Dulbecco's modified Eagle's medium (DEM) kept in a 10% CO₂ incubator. *a*, Untreated control, resting secondary chick cells prepared by seeding 4×10^6 cells per 35-mm dish in DEM+0.5% calf serum (Grand Island Biological) for 3 d; *b*, trypsin at $0.2 \mu\text{g ml}^{-1}$ for 4 h; *c*, trypsin at $0.1 \mu\text{g ml}^{-1}$ for 4 h; *d*, insulin at $5 \mu\text{g ml}^{-1}$ for 4 h; *e*, insulin at $1 \mu\text{g ml}^{-1}$ for 4 h; *f*, thrombin at $50 \mu\text{g ml}^{-1}$ for 30 min; *g*, thrombin at $20 \mu\text{g ml}^{-1}$ for 4 h; *h*, thrombin at $2 \mu\text{g ml}^{-1}$ for 24 h; *i*, thrombin at $1 \mu\text{g ml}^{-1}$ for 2 h. Washed cells were iodinated in 1 ml of Dulbecco's phosphate-buffered saline containing 0.9 mM CaCl₂ and 0.9 mM MgCl₂ with addition of 20 μg of lactoperoxidase (Worthington) further purified by chromatography on a Sephadex G-100 column, 50 μCi of carrier-free Na¹²⁵I (New England Nuclear) in 0.1 N NaOH, and 2.4 nmol of NaI. The reaction was initiated by addition of 5 μmol of glucose and 0.015 U of glucose oxidase (Sigma). Reactions were terminated after 15 min by five washes of ice-cold Ca²⁺- and Mg²⁺-free phosphate-buffered saline solution. The cells were then dissolved in electrophoresis sample buffer containing 2% SDS, 1% mercaptoethanol and 2 mM phenylmethane sulphonyl fluoride. Slab gel electrophoresis was carried out as described previously² except that the acrylamide gradient was 6–15%. The radioactivity loaded on a slab gel was 20,000 c.p.m. for each sample. The slab gel was dried and subjected to autoradiography on Kodak RP Royal X-Omat X-ray film for 3–24 h. The standards used for molecular weight determination are myosin, β -galactosidase, bovine serum albumin, actin and chymotrypsinogen.

of iodlatable proteins, many more than in previous results^{2-4,7-10}. Most of the bands in the autoradiogram are sharp and well defined compared with other reports where ¹²⁵Iodide was used. We find that one band (indicated by an arrow) is sensitive to thrombin. Column *f* shows that after treating the cells for 30 min with a high concentration of thrombin ($50 \mu\text{g ml}^{-1}$) this protein can no longer be detected when compared with the external proteins from a control culture of resting chick cells (column *a*).

The experimental conditions for columns *g*, *h* and *i*, respectively, are: resting chick cells treated with thrombin at $20 \mu\text{g ml}^{-1}$ for 4 h, $2 \mu\text{g ml}^{-1}$ for 24 h and $1 \mu\text{g ml}^{-1}$ for 2 h. A significant observation has been that in all these conditions one iodlatable protein of molecular weight 205,000 was consistently sensitive to thrombin. Figure 2 shows microdensitometer scans of two of the gel strips (*a* and *g*) of Fig. 1. These scans confirm quantitatively the absence of only one iodlatable cell surface protein after treatment of chick embryo fibroblasts with thrombin. This thrombin-sensitive protein, as expected, is also sensitive to trypsin.

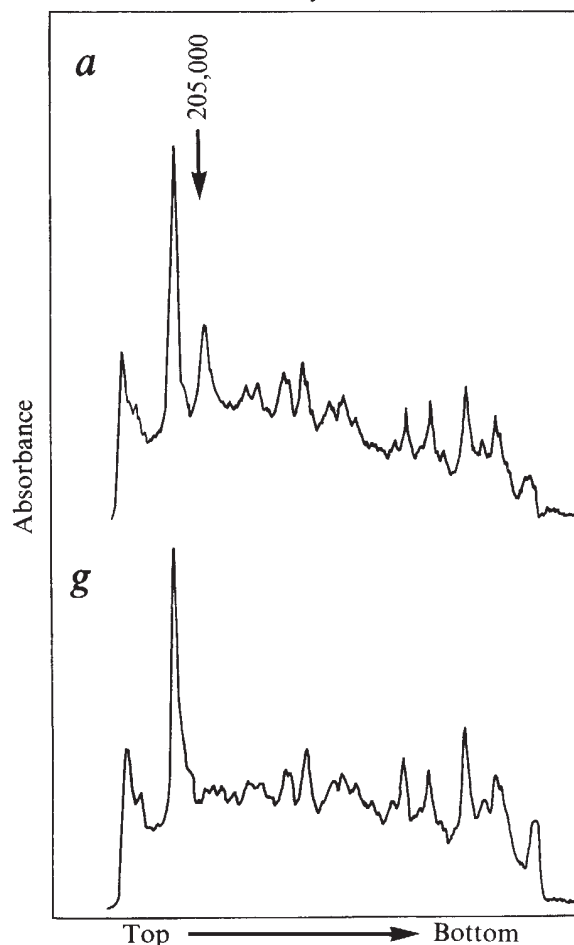
As in the previous report where ¹²⁵Iodide was used, we find that LETS protein is not removed by thrombin treat-

ment². LETS protein stands out even more clearly as the most prominent cell surface protein in cases where ¹³¹Iodine was used in place of ¹²⁵Iodide. As columns *b* and *c* show, both LETS and the 205,000 protein were removed by trypsin. On the other hand, insulin at $1 \mu\text{g ml}^{-1}$ or $5 \mu\text{g ml}^{-1}$ for 4 h does not remove thrombin-sensitive protein (columns *d* and *e*).

When serum proteins were labelled with ¹³¹Iodine by lactoperoxidase-catalysed reaction, no iodlatable protein comigrating with the thrombin-sensitive protein was detected. Furthermore, the availability of this thrombin-sensitive protein for iodination on the cell surface is not serum dependent. Chick embryo cells passaged in serum-free medium (but supplemented with insulin at $5 \mu\text{g ml}^{-1}$) have the same amount of thrombin-sensitive protein as that of cells grown in 10% serum. Finally, sparse, exponentially growing chick cells incubated in 10% serum have only a very small amount of thrombin-sensitive protein. Therefore, it is unlikely that this protein is a component of serum. Since it has been established⁹⁻¹⁰ that lactoperoxidase-catalysed iodination labels only proteins on the surface of cells, this thrombin-sensitive protein is probably an external cell-surface protein. The possibility that this thrombin-sensitive protein is released by dead cells in culture is ruled out because we used only cultures of secondary chick cells, which have no detectable. Trypan blue-positive cells both before and after iodination.

The role of the 205,000 protein is unknown. It is possible

Fig. 2 Densitometric scans of gel strips *a* and *g* of Fig. 1. Two of the autoradiograms shown in Fig. 1 (*a* and *g*) were scanned by a Canalco Model G 11 densitometer with a digital integrator (Autolab 6300) attachment. Since the thrombin-treated cultures (*f*–*i*) gave essentially the same scanning results only *g* was shown in this figure. The scan of gel strip *a* represents the untreated control. Thrombin-sensitive proteins are indicated by an arrow.



that thrombin attacks the 205,000 protein proteolytically and that this event triggers renewed cell proliferation. Alternatively, the effect of thrombin on the resting cell might be stimulation of cell proliferation by some unknown mechanism concerned with altered cell-cell interaction in the absence of the external 205,000 protein. These two possibilities are being investigated.

Cell-surface proteins (including membrane proteins and surface coat proteins) are undoubtedly important in many complex biological phenomena. Progress in these studies has been slow because the role of a surface protein is always difficult to establish. An ideal way for the assignment of biological function to a surface component is the use of cell mutants lacking a specific protein(s). Alternatively, if a protease probe is available that can specifically alter one surface protein, the assignment of its function may be achieved by comparison with a control culture in which a protease has not been included. Use of highly specific proteases such as those involved in blood coagulation, for example, thrombin, factor X_a and IX_a, may prove to be fruitful for such work.

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NELSON N. H. TENG*
LAN BO CHEN

Department of Biology,
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Received October 1; accepted December 18, 1975.

*Present address: Department of Pharmacology, School of Medicine, University of Miami, Miami, Florida 33152.

- 1 Chen, L. B., and Buchanan, J. M., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 131-135 (1975).
- 2 Teng, N. N. H., and Chen, L. B., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 413-417 (1975).
- 3 Chen, L. B., Teng, N. N. H., and Buchanan, J. M., *Proteases and Biological Control* (edit. by Reich, E., Rifkin, D., and Shaw, E.), 957-965 (Cold Spring Harbor Laboratory, New York, 1975).
- 4 Magnusson, S., *The Enzymes* (edit. by Boyer, P. D.), **3**, 277-321 (Academic, New York, 1971).
- 5 Muszbek, L., and Laki, K., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2208-2211 (1974).
- 6 Hynes, R. O., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3170-3174 (1973).
- 7 Hogg, N. M., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 489-492 (1974).
- 8 Wickus, G. G., Branton, P. E., and Robbins, P. W., *Control of Proliferation in Animal Cells* (edit. by Clarkson, B., and Baserga, R.), 541-546 (Cold Spring Harbor Laboratory, New York, 1974).
- 9 Stone, K. R., Smith, R. E., and Joklik, W. K., *Virology*, **58**, 86-100 (1974).
- 10 Hynes, R. O., *Cell*, **1**, 147-156 (1974).
- 11 Blumberg, P. M., and Robbins, P. W., *Proteases and Biological Control* (edit. by Reich, E., Rifkin, D., and Shaw, E.), 945-956 (Cold Spring Harbor Laboratory, New York, 1975).

New structural characteristic of the large glycopeptides from transformed cells

IN comparative gel filtration of glycopeptides obtained from the surface of normal and transformed cells, an increased concentration of fucosyl glycopeptides of apparently large molecular weight was observed for cells transformed by viral, chemical and spontaneous methods¹⁻⁷. This is considered to be an expression of transformed phenotype, since in cells transformed by a temperature-sensitive (*ts*) mutant of Rous sarcoma virus, the heavy glycopeptides increased only at the permissive temperature. Based on the results of sialidase treatment, it was suggested that the presence of extra sialic acid is responsible for the higher molecular weight nature of the glycopeptides from transformed cells^{4,8}.

In this communication, however, we present evidence that the differences are not that simple, but are more

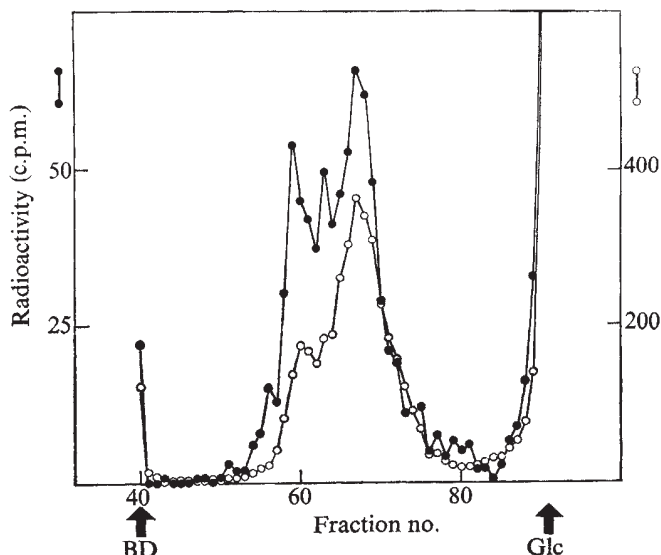


Fig. 1 Sephadex G-50 column chromatography of fucose-labelled glycopeptides from trypsinates of normal and polyoma transformed cells. BHK₂₁₋₁₁ cells and the cells transformed by polyoma virus were grown at 37 °C in plastic Petri dishes (growth area 78 cm²) containing 10 ml of Eagle's MEM supplemented with 10% foetal calf serum and antibiotics in a humidified CO₂ incubator. The inoculum was 1 × 10⁶ cells per dish. Three days after plating, 30 µCi of ³H-fucose (4.4 Ci mmol⁻¹) or 3 µCi of ¹⁴C-fucose (50.8 mCi mmol⁻¹) was added to each dish. After 24 h, the cultures were washed twice with Earle's solution, and treated with 5 ml of 0.1% trypsin (Difco) in Earle's solution lacking CaCl₂ and MgSO₄ and containing 0.016% EDTA for 5 min at room temperature. Then the medium was carefully decanted and centrifuged at 500g for 5 min to remove any loose cells. The supernatant (termed as 'trypsinates') was digested with Pronase for 3 d adding 1 mg ml⁻¹ of Pronase every day. Then, Pronase was inactivated by heating at 100 °C for 5 min, and the digests were applied to a column of Sephadex G-50, fine (1.0 × 125 cm). The column was equilibrated and eluted with 0.1 M pyridine-acetate buffer, pH 5.5. Fractions (1 ml) were collected. Aliquots (0.3 ml) were removed for counting, and the rest of the material was pooled as the large glycopeptides (fraction 56 to 62) and the small glycopeptides (fraction 64 to 74), lyophilised and stored. The figure shows a typical double labelling experiment in which the glycopeptides from BHK cells (○) were labelled with ¹⁴C and those from polyoma-transformed BHK cells (●) were labelled with ³H. The standard glycopeptides, (Man)₆(GlcNAc)₂N-(¹⁴C)acetyl-Asn (molecular weight 1,393), ¹⁴C-acetylated bovine IgG glycopeptides (molecular weight ~2,000) and ¹⁴C-acetylated unit B glycopeptides of thyroglobulin (molecular weight ~3,000) were eluted in fractions 77-78, 72 and 66, respectively. Molecular weight of the large and the small glycopeptides was estimated by the method of Andrews¹⁸.

extensive, possibly including both galactose and N-acetylglucosamine.

The fucose-labelled large glycopeptides were prepared from the trypsinates of polyoma-transformed BHK cells, and the small glycopeptides from the trypsinates of the normal and the polyoma-transformed cells (Fig. 1). In agreement with the work of Warren *et al.*^{3,4}, the large glycopeptides were more enriched in transformed cells. On paper electrophoresis at pH 5.4, the large glycopeptides migrated faster to the positive pole than the small glycopeptides. Neuraminidase treatment of these glycopeptides converted most of them into neutral glycopeptides, indicating that the negative charge of these glycopeptides arose from sialic acid. We could also, therefore, confirm that the large glycopeptides have more sialic acids than the small glycopeptides.

These glycopeptides were studied comparatively by affinity column chromatography on concanavalin A (con A)-Sephrose⁸. About 70% of the small glycopeptides from both normal and polyoma-transformed cells were bound to the column. Most of the bound glycopeptides

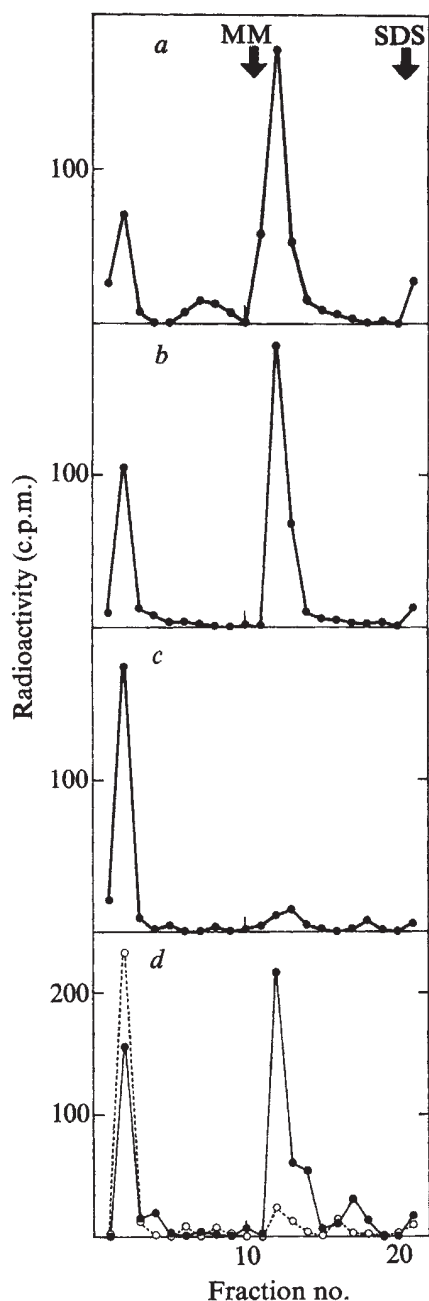


Fig. 2 Affinity column chromatography of glycopeptides on con A-Sepharose. *a*, The small glycopeptides from BHK cells; *b*, the small glycopeptides from polyoma-transformed BHK cells; *c*, the large glycopeptides from polyoma-transformed BHK cells, and *d*, the large glycopeptides from polyoma-transformed BHK cells treated with neuraminidase alone (·····) or with neuraminidase, β -galactosidase and β -*N*-acetylglucosaminidase (—). Glycopeptides and their derivatives were applied to a column of con A-Sepharose (Pharmacia, Sweden) equilibrated with 0.01 M Tris-HCl buffer, pH 7.5 containing 0.1 M NaCl. The column was washed with 10 ml of the above buffer, and then eluted with 10 ml of the same buffer containing 0.1 M methyl α -mannoside. Fractions (1 ml) were collected. Then con A-Sepharose was extracted with 5 ml of 1% SDS at 100 °C for 2 min. Glycopeptides in 0.02 ml of 0.1 M phosphate buffer, pH 6.0 were mixed with 0.01 ml of neuraminidase from *Clostridium perfringens* (0.01 units, Sigma) and were incubated at 37 °C for 2 h. The neuraminidase-treated glycopeptides were heated at 100 °C for 5 min and adjusted to pH 4.0 by the addition of 0.01 ml of 0.15 M citrate-phosphate buffer, pH 3.0. Then, 0.04 ml each of β -galactosidase¹⁷ (0.64 U) and β -*N*-acetylglucosaminidase¹⁸ (3.7 U) from jack bean meal were added and the mixture was incubated at 37 °C for 16 h with a small amount of toluene.

were eluted by 0.1 M methyl α -mannoside, and a small amount of tightly bound glycopeptides by 1% sodium dodecyl sulphate (SDS) (Fig. 2 *a*, *b*). In contrast, we found that only 20% of the large glycopeptides from polyoma-transformed cells were bound to the column (Fig. 2*c*). This behaviour of the large glycopeptides did not change, even after neuraminidase digestion (Fig. 2*d*, dotted line), indicating that the enrichment of sialic acid is not responsible. A new structural difference between the large and the small glycopeptides was therefore shown.

We then compared core structures of the large glyco-

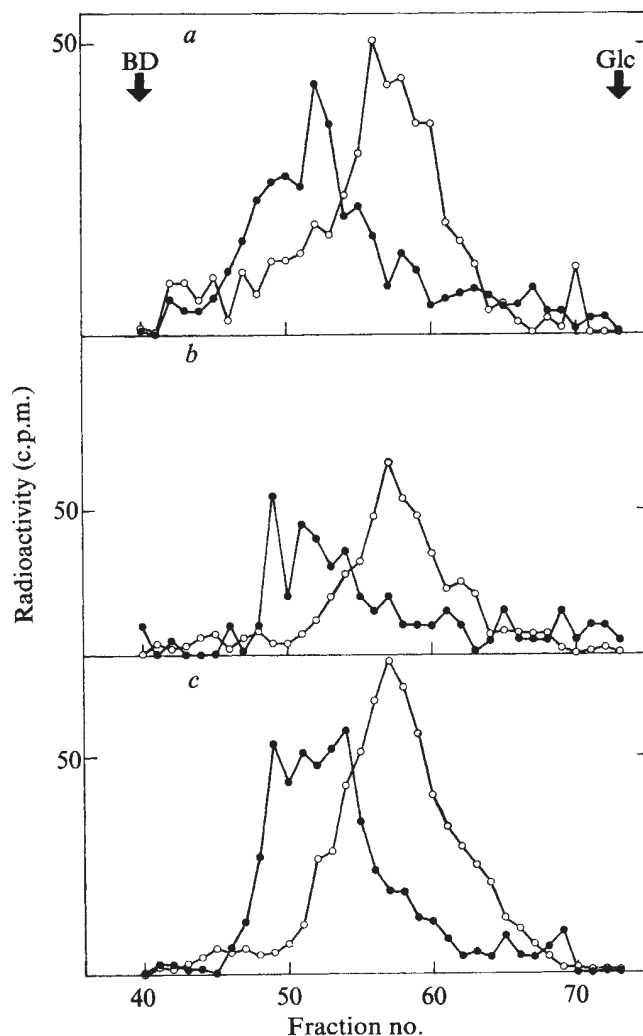


Fig. 3 Sephadex G-25 column chromatography of glycopeptides treated with glycosidases. *a*, The large glycopeptides from polyoma-transformed BHK cells; *b*, the small glycopeptides from polyoma-transformed BHK cells; *c*, the small glycopeptides from normal BHK cells. Chromatography was performed on a column of Sephadex G-25, fine (1 $\times$ 125 cm) equilibrated and eluted with 0.01 M Tris-HCl buffer, pH 7.5 containing 0.1 M NaCl. Fractions of 1 ml were collected. The standard substances, (Man)₃(GlcNAc)₂*N*-(¹⁴C)-acetyl-Asn (molecular weight 1,393) and GlcNAc-*N*-(¹⁴C)-acetyl-Asn (molecular weight 379) were eluted in fractions 47–48 and 60–61, respectively. ●, Glycopeptides treated with neuraminidase, β -galactosidase and β -*N*-acetylglucosaminidase. The condition of exoglycosidase digestion was the same as described in Fig. 2. ○, Glycopeptides treated with endo- β -*N*-acetylglucosaminidase D in the presence of neuraminidase, β -galactosidase and β -*N*-acetylglucosaminidase. The glycopeptides were digested with the mixture of 10.4 milliunits of endo- β -*N*-acetylglucosaminidase D (ref. 9), 5.8 mU of neuraminidase¹⁹, 14.2 mU β -galactosidase¹⁹ and 46 mU β -*N*-acetylglucosaminidase²⁰, all from *Diplococcus pneumoniae* in 0.06 ml of 0.05 M citrate-phosphate buffer, pH 6.6 at 37 °C for 16 h. The products of the endoglycosidase digestion were concluded to be glycopeptide fragments and not oligosaccharides, since they migrated identically with the glycopeptide fragments from IgG glycopeptides on paper chromatography in butanol-acetic acid–1 N NH₄OH (2:3:1).

peptides and the small glycopeptides. The cores were prepared by enzymatic removal of sialic acid, galactose and *N*-acetylglucosamine. We found that 70% of the cores both from the large glycopeptides of transformed cells (Fig. 2d, solid line) and from the small glycopeptides of normal and transformed cells (data not shown) were bound to the column of con A-Sepharose. Molecular weights of the cores were estimated to be $\sim 1,100$ in all the cases (Fig. 3). Further similarity of core structures was indicated by digestion with endo- β -*N*-acetylglucosaminidase D (ref. 9). Most of the large and the small glycopeptides were hydrolysed by endo- β -*N*-acetylglucosaminidase D in the presence of neuraminidase, β -galactosidase and β -*N*-acetylglucosaminidase, yielding glycopeptide fragments of molecular weight ~ 450 (Fig. 3). Therefore, in both the large and the small glycopeptides, fucose residue was located in the vicinity of protein-carbohydrate linkage region as described previously in human diploid cells¹⁰. In addition to the linkage region, clusters of mannosyl residues must be present in the cores of most of the glycopeptides, since both susceptibility to endo- β -*N*-acetylglucosaminidase D (ref. 11) and binding to con A-Sepharose⁸ required their presence. The number of the mannosyl residues in the glycopeptides were estimated to be ~ 3 in all the cases, since the difference of molecular weights between the core and the glycopeptide fragments was ~ 650 , and the value roughly corresponded to the molecular weight of Man₃GlcNAc, the principal oligosaccharide released from cell-surface glycopeptides by endo- β -*N*-acetylglucosaminidase D (ref. 12). Therefore, cores of the large and the small glycopeptides were very similar to each other and to those of non-membrane glycopeptides such as IgG glycopeptides^{13,14} (Fig. 4).

Summarising the results, the large glycopeptides and the small glycopeptides were different in their behaviour towards con A-Sepharose in spite of the close identity of the core structures. We have previously indicated that the presence of two α -mannosyl residues capable of interacting with con A is required for a glycopeptide to bind to the column⁸. The interacting α -mannosyl residues are those with free hydroxyl groups on C3, C4 and C6 (ref. 15). Thus, the large glycopeptides may have more branches composed of sialic acids, galactose and *N*-acetylglucosamine (Fig. 4). These additional branches will take the place of the hydroxyl groups of α -mannosyl residues, resulting in the reduction of the number of α -mannosyl residues interacting with con A.

The above conjecture is also supported by molecular weight values of the two glycopeptides. Calibrating the Sephadex G-50 column with standard glycopeptides, we could estimate approximate molecular weights of the large and the small glycopeptides to be 4,000 and 2,500, respectively (Fig. 1). Thus, the molecular weight difference of the large and the small glycopeptides was 1,500, which might be too large to be explained by the addition of sialic acid itself, but can be easily explained by the addition of whole chains of sialyl-galactosyl-*N*-acetylglucosaminyl

branches (Fig. 4). If the above inference is correct, the key step leading to the large glycopeptides is not sialylation⁴, but the initial formation of additional branches, possibly *N*-acetylglucosaminylation.

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SHUN-ICHIRO OGATA
TAKASHI MURAMATSU
AKIRA KOBATA

Department of Biochemistry,
Kobe University School of Medicine,
Ikuta-ku, Kobe, Japan

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- ¹ Buck, C. A., Glick, M. C., and Warren, L., *Biochemistry*, **9**, 4567-4576 (1970).
- ² Buck, C. A., Glick, M. C., and Warren, L., *Science*, **172**, 169-171 (1971).
- ³ Warren, L., Critchley, D., and Macpherson, I., *Nature*, **235**, 275-277 (1972).
- ⁴ Warren, L., Fuhrer, J. P., and Buck, C. A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1838-1842 (1972).
- ⁵ Buck, C. A., Fuhrer, J. P., Soslaw, G., and Warren, L., *J. biol. Chem.*, **249**, 1541-1550 (1974).
- ⁶ Van Beek, W. P., Smets, L. A., and Emmelot, P., *Cancer Res.*, **33**, 2913-2922 (1973).
- ⁷ Van Beek, W. P., Smets, L. A., and Emmelot, P., *Nature*, **253**, 457-460 (1975).
- ⁸ Ogata, S., Muramatsu, T., and Kobata, A., *J. Biochem., Tokyo*, **78**, 687-696, (1975).
- ⁹ Koide, N., and Muramatsu, T., *J. biol. Chem.*, **249**, 4897-4904 (1974).
- ¹⁰ Muramatsu, T., Atkinson, P. H., Nathanson, S. G., and Ceccarini, C., *J. molec. Biol.*, **80**, 781-799 (1973).
- ¹¹ Ito, S., Muramatsu, T., and Kobata, A., *Biochem. biophys. Res. Commun.*, **63**, 938-944 (1975).
- ¹² Muramatsu, T., Koide, N., and Ogata-Arakawa, M., *Biochem. biophys. Res. Commun.*, **66**, 881-888 (1975).
- ¹³ Kornfeld, R., Keller, J., Baenziger, J., and Kornfeld, S., *J. biol. Chem.*, **246**, 3259-3268 (1971).
- ¹⁴ Tai, T., Ito, S., Yamashita, K., Muramatsu, T., and Kobata, A., *Biochem. biophys. Res. Commun.*, **65**, 968-973 (1975).
- ¹⁵ Goldstein, I. J., Hollerman, C. E., and Smith, E. E., *Biochemistry*, **4**, 876-883 (1965).
- ¹⁶ Andrews, P., *Biochem. J.*, **91**, 222-233 (1964).
- ¹⁷ Arakawa, M., Ogata, S., Muramatsu, T., and Kobata, A., *J. Biochem., Tokyo*, **75**, 707-714 (1974).
- ¹⁸ Li, Y.-T., and Li, S.-C., *Meth. Enzym.*, **28**, 702-713 (1972).
- ¹⁹ Hughes, R. C., and Jeanloz, R. W., *Biochemistry*, **3**, 1535-1543 (1964).
- ²⁰ Hughes, R. C., and Jeanloz, R. W., *Biochemistry*, **3**, 1543-1548 (1964).

Influenza A antigens on human lymphocytes *in vitro* and probably *in vivo*

INFLUENZA A viruses cause respiratory tract infections in man, associated with generalised symptoms of fever, headache and myalgia. Virus replication seems to be limited to the respiratory tract epithelium¹, and attempts to demonstrate a viraemia have been largely unsuccessful², although it is not unknown³. The mechanisms producing the generalised symptoms are not yet understood. In this paper we demonstrate the probable presence of viral antigens on peripheral blood lymphocytes from patients during peak infection with influenza. This could have important implications both for the pathogenesis of influenza and for early diagnosis.

The technique we used was the mixed antiglobulin rosette-forming reaction⁴ (MAR), which involved two stages of lymphocyte sensitisation. The lymphocytes to be tested for adsorbed influenza antigens were first treated for 45 min at 4 °C with rabbit anti-influenza serum (R9 or R18; see footnote to Table 1), or with normal rabbit serum (NRS) or diluent as controls. All rabbit sera had been heat inactivated and absorbed $\times 3$ with human erythrocytes and $\times 3$ with human formalised leukaemic spleen cells to remove anti-species antibodies. The lymphocytes were washed twice after the first-stage sensitisation, and then exposed to a sheep (207, bleed 5) anti-rabbit globulin serum (45 min, 4 °C). They were washed twice more and centrifuged (350g, 2 min, 4 °C) for rosette formation with equal volumes (20-25 μ l) of 0.8% (v/v) sheep erythrocytes coated with immunoglobulin from a rabbit anti-sheep erythrocyte serum (R8104/II, dilution 1:10,000). The pelleted cells were gently resuspended and mixed with toluidine blue stain for microscopic examination.

Lymphocytes for examination were separated from

Fig. 4 A structural model of the majority of the large glycopeptides from transformed cells. The number of branches was estimated to be ~ 4 , since the molecular weight difference of the intact glycopeptides and the core was $\sim 2,900$. In the small glycopeptides, the core structure was probably identical, but the number of branches was considered to be smaller.

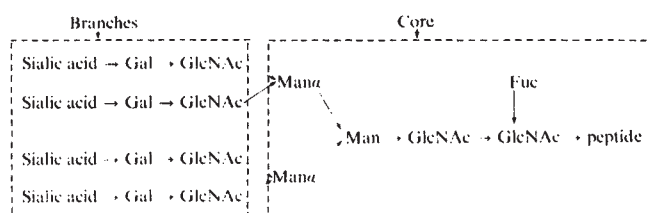


Table 1 Percentages of lymphocytes from six normal asymptomatic humans reacting in the MAR

Lymphocytes (formaldehyde treated) $2.5 \times 10^6 \text{ ml}^{-1}$	Serum HI titre	Serum used in first stage of sensitisation*						PBS/BSA controls
		R9 anti-MRC11 1:25	1:100	R18 anti-MRC2 1:25	1:100	NRS 1:25	1:100	
J.H.	10	2.5	2	3.5	3	4	3.5	2
Z.M.	10	5	3.5	2	0	1.5	2	2
K.A.	10	4	4	4	5	3	4	2
D.F.	10	3.5	4.5	5	4.5	2	2	0
M.F.	20	2	2	7	3	2	1.5	0
E.M.	10	3	2	6	5	5	4.5	2

* Rabbit anti-influenza sera R9 raised against MRC11 recombinant strain, and R18 raised against MRC2 strain, both virus strains closely related antigenically to current strains with surface properties H_3N_2 .

heparinised blood by treating with carbonyl iron, followed by differential centrifugation on Ficoll-Hypaque⁵. The purified lymphocyte suspension was washed twice with phosphate-buffered saline (PBS) and treated with formaldehyde⁶ to prevent the formation of Fc and E rosettes with the indicator cells used later in the MAR. The fixed cells were washed twice with PBS and stored in sterile bottles at 4 °C until tested, at which stage they were washed twice more and resuspended to approximately $2.5 \times 10^6 \text{ cells ml}^{-1}$ in 0.2% bovine serum albumin in PBS (PBS/BSA).

Preliminary experiments were carried out on blood lymphocytes from healthy individuals and the results compared with those on normal lymphocytes which had been

agent in NRS is antibody immunoglobulin which is able to combine which one or more antigenic components of influenza A. This conclusion was supported by further experiments in which the reactivity of NRS was retained, and actually increased, after treatment to remove nonspecific haemagglutination inhibiting factors⁷ by (1) heating to 65 °C for 30 min, (2) treating with potassium periodate, (3) heating as in (1) plus periodate treatment, or (4) trypsin digestion.

The patients providing lymphocytes to be tested for *in vivo* adsorbed virus were selected by medical general practitioners on a clinical diagnosis of influenza. Between 24 h and 5 d after onset of symptoms, blood was taken, heparinised for lymphocyte separation, and clotted for

Table 2 Percentages of reacting cells in MAR given by formalised normal human lymphocytes untreated, treated with 'split' influenza MRC11 strain virus*, and treated with whole influenza MRC11 virus suspension†

Serum used in first stage of sensitisation	Lymphocytes untreated‡	Lymphocytes treated with split virus		Lymphocytes treated with whole virus ($\approx 40 \mu\text{g viral protein ml}^{-1}$)
		1/200 ($\approx 10 \mu\text{g viral protein ml}^{-1}$)	1/2,000 ($\approx 1 \mu\text{g viral protein ml}^{-1}$)	
R9 anti-MRC11 1:25	2 (7)	98	61	93
1:100	3.5	99	49	95
R18 anti-MRC2 1:25	3 (6)	99	NT	98
1:100	1.5	98	NT	97
NRS pool 1:25	5 (5.5)	93	37	82
1:100	2	71	11	76
PBS/BSA control	1 (3)	13	3.5	23

* Lymphocytes ($2.5 \times 10^6 \text{ cells ml}^{-1}$) were mixed with split virus products, obtained by ether treatment of whole virus, at room temperature for 45 min. Cells were then washed twice before formaldehyde fixation.

† Whole virus purified from culture in allantoic fluid of hens' eggs was added to the lymphocyte suspension (room temperature 45 min) which was then washed and fixed as described above.

‡ Figures in parentheses are results for untreated control cells in test on lymphocytes exposed to whole virus.

NT, Not tested.

exposed *in vitro* to either whole MRC11 strain (influenza recombinant H_3N_2) virus suspension or ether-treated MRC11 split viral products (see footnote to Table 2). Lymphocytes from six normal individuals showed low percentages of reacting cells—usually 5% or fewer with either anti-influenza serum or NRS (Table 1). There were significant increases in percentages of rosetting lymphocytes, with up to 100% of cells reacting, when the lymphocytes were exposed to either whole MRC11 viruses or ether-treated split virus before formaldehyde fixation (Table 2). The reactivity, however, was also markedly increased, though to a somewhat lesser extent, in suspensions treated with NRS. A second pool of NRS and rabbit R9 pre-inoculation serum exhibited similar reactivity. When doubling dilutions of NRS (pool 23/4/70 and 30/6/70) and R18 anti-influenza serum were tested on lymphocytes coated with (1:100) split viral products, lower reactions were produced with the NRS, so that at 1:1,600 dilution R18 antiserum gave 92% rosettes while NRS gave only 31% weak rosettes. The reactive factor was also present in DEAE-cellulose purified rabbit IgG fraction, and F(ab')_2 fragments of pepsin-digested rabbit IgG also reacted (Table 3). Since the uptake on to lymphocytes of rabbit serum proteins other than immunoglobulin would not be detected by the two-stage MAR, all the evidence suggests that the reactive

serum extraction. To confirm diagnosis, a throat swab was cultured and identification of the infecting agent attempted. A second serum sample was taken 10 d to 3 months after the onset, and both sera were tested by haemagglutination inhibition (HI) for any rise in anti-influenza A antibodies. The results of these tests are shown in Table 4.

Influenza A was grown from three of the ten patients and two more had serological evidence of infection. In four of the five positive patients the percentage of lymphocytes giving reactions for surface-bound influenza antigens

Table 3 Percentages of reacting cells in MAR on normal human lymphocytes, untreated and treated with 1/20 diluted 'split' virus, to show reactions with NRS, DEAE-cellulose purified rabbit IgG and rabbit IgG pepsin F(ab')_2

Reagent used in first stage of sensitisation	Dilution/concentration	Lymphocytes untreated	Lymphocytes treated with 1/20 split virus
NRS pool	1:25	7	85
NRS IgG	350 $\mu\text{g ml}^{-1}$	7	89
NRS IgG F(ab')_2	320 $\mu\text{g ml}^{-1}$	4	40
PBS/BSA control		2	11

Table 4 Tests on patients with symptoms of influenza infection. Results of throat swab cultures, serum antibody titres measured by haemagglutination inhibition (HI) and percentages of lymphocytes in the MAR

Patient	Swab culture	Serum HI titre*	Mixed antiglobulin reaction of lymphocytes† following first stage sensitisation with		
			R9 anti-MRC11 (1/25)	R18 anti-MRC2 (1/25)	NRS pool (1/25)
R.B.	—	10 40	22	26	24
R.T.	+ ('A' Port Chalmers)	10 80	20	28	14
A.N.	+ ('A' Scotland)	10 1,280	45	NT	29
P.S.	No swab	10 80	19	16	12
P.H.	+ ('A' Port Chalmers)	<10 640	3	3	1.5
L.A.S.	—	<10 10	2.5	1	1
J.M.	—	<10 10	2	2	2
H.T.P.	No swab	<10 20	9	4	4.5
J.D.A.	— (but + for respiratory syncytial virus)	20 160	4	7.5	6
J.J.B.	—	320 320	10	2	7

* For each patient, the first figure is for serum collected at the same time as blood for lymphocytes, and the second for serum taken 10 d to 3 months later.

† These results were obtained 'blind', since the results of swab cultures and HI serum titres were unknown to the tester.

NT, Not tested.

was substantially greater than for the remainder of the group, in which the reactions were mainly similar to those of lymphocytes from normal, non-infected individuals. The highest proportion of reacting lymphocytes (45%) was found in a patient (A.N.) infected with influenza A Scotland 74 serotype, and he also showed the highest antibody titre (HI 1:1,280, 2 months after infection). Patient R. B., with 22% to 26% reacting lymphocytes, gave a negative culture, but the specific increase in serum HI titre (3 months postinfection) indicates that the infection was probably influenza.

The fifth influenza-positive patient (P.H.) gave a negative MAR test, although the virus had been grown and antibody production demonstrated. The lymphocytes from this person were obtained within 24 h of the onset of symptoms, whereas those from other positive patients were obtained 2–5 d after the onset. It is thus possible that there is a delay of at least 24 h between the onset of disease and the appearance of detectable viral antigen on circulating lymphocytes. It will be seen that, as with lymphocytes coated with viral antigens *in vitro*, lymphocytes from positive patients also reacted with NRS, although the numbers of rosetted cells found in three out of the four cases were lower than with anti-influenza serum.

The binding and subsequent inactivation of influenza and other myxoviruses by cultures of human and non-immunised, pathogen-free mouse lymphoid cells has previously been demonstrated^{8,9}. Other viruses also known to adhere to the lymphocyte cell membrane are the Epstein-Barr virus, which attaches to B lymphocytes but not to T cells¹⁰ and the measles virus, receptors for which are found only on T lymphocytes¹¹. It would seem from the present work that influenza A will adhere *in vitro* to both T and B cells since < 100% of cells were positive in the MAR test. Fewer cells with attached virus were found in the peripheral blood of patients, however, and it is not yet known whether there is any bias in the *in vivo* adherence in favour of T or B cells.

Further investigations on influenza infection in ferrets have been initiated and it is hoped that these will provide information on the nature and spread of lymphocyte-adherent viral components in better controlled conditions than are possible in human infections.

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ANNE B. WILSON
D. N. PLANTEROSE
J. NAGINGTON
JENNIFER R. PARK
R. D. BARRY
R. R. A. COOMBS

*Divisions of Immunology and Virology,
Department of Pathology, University of Cambridge, and
Public Health Laboratory Service,
Laboratories' Block,
Addenbrooke's Hospital,
Hills Road, Cambridge CB2 2QQ, UK*

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- Denman, A. M., and Pinder, Margaret M. A., *Proc. R. Soc. Med.*, **67**, 1219–1221 (1974).
- Mackenzie, J. S., and Houghton, M., *Bact. Rev.*, **38**, 356–370 (1974).
- Naficy, K., *New Engl. J. Med.*, **259**, 964–966 (1963).
- Coombs, R. R. A., and Franks, D., *Prog. Allergy*, **13**, 174–272 (1969).
- Böyum, A., *Scand. J. clin. Lab. Invest.*, **21**, suppl. 97–106 (1968).
- Hallberg, T., et al., *J. immun. Meth.*, **4**, 317–332 (1974).
- Hoyle, L., *The Influenza Viruses*, Virology Monograph, **4** (Springer, Vienna, 1968).
- Zisman, B., and Denman, A. M., *J. gen. Virol.*, **20**, 211–233 (1973).
- Hackemann, Margaret M. A., Denman, A. M., and Tyrrell, D. A. J., *Clin. exp. Immun.*, **16**, 583–591 (1974).
- Jondal, M., and Klein, G., *J. exp. Med.*, **138**, 1365–1378 (1973).
- Valdimarsson, H., Agnarsdottir, G., and Lachmann, P. J., *Nature*, **255**, 554–556 (1975).

Dopamine-like renal and mesenteric vasodilation caused by apomorphine 6-propylnorapomorphine and 2-amino-6, 7-dihydroxy-1,2,3,4-tetrahydronaphthalene

DOPAMINE has several unique actions in the central nervous system (CNS)—particularly the striatum—and in blood vessels, which seem to be due to interactions with specific receptors^{1,2}. It is not known whether the vascular and CNS effects are mediated by actions on the same or different receptors. To demonstrate receptor identity, classical receptor theory requires that tissues respond in the same

rank order to a series of agonists of varying potency³. Remarkable similarities have been revealed in the relative efficacy of dopamine agonists in dilating canine renal blood vessels⁴ and in increasing dopamine-sensitive cyclic AMP accumulation in homogenates of rat striatum⁵. Further characterisation has been restricted for lack of additional agonists to extend the potency series. We describe here a comparison of the actions of two recently described dopamine agonists, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) and 6-propyl-norapomorphine (PNA) with dopamine and apomorphine in the canine renal and mesenteric vascular beds. Apomorphine, which has structural similarities to dopamine, causes dopamine-like vasodilation⁴ and increases cyclic AMP accumulation in rat striatum⁵ but is much less potent and efficacious than dopamine in both systems. Woodruff *et al.*⁶ found that ADTN exerted dopamine-like actions in the CNS of mice and resembled dopamine in decreasing blood pressure in guinea pigs. ADTN is equipotent with dopamine in increasing cyclic AMP accumulation in rat striatal homogenates⁵, and intra-striatal injections of ADTN in rats and guinea pigs produce a dyskinetic phenomenon characteristic of dopamine-like agents^{7,8}. Cannon^{9,10} suggested that PNA was a dopamine agonist on the basis of its emetic potency in the anaesthetised dog.

ADTN and PNA were chosen after preliminary experiments suggested that they have dopamine-like vasodilating actions. Methods were similar to those described before¹¹. Mongrel dogs were anaesthetised with pentobarbital (32 mg kg⁻¹) given intravenously. Anaesthesia was maintained by injections of 32 or 64 mg of pentobarbital when required. Blood flows in the renal, mesenteric and femoral arteries were monitored continuously by a Statham electromagnetic flowmeter coupled to a Beckman Dynograph. Vasodilating

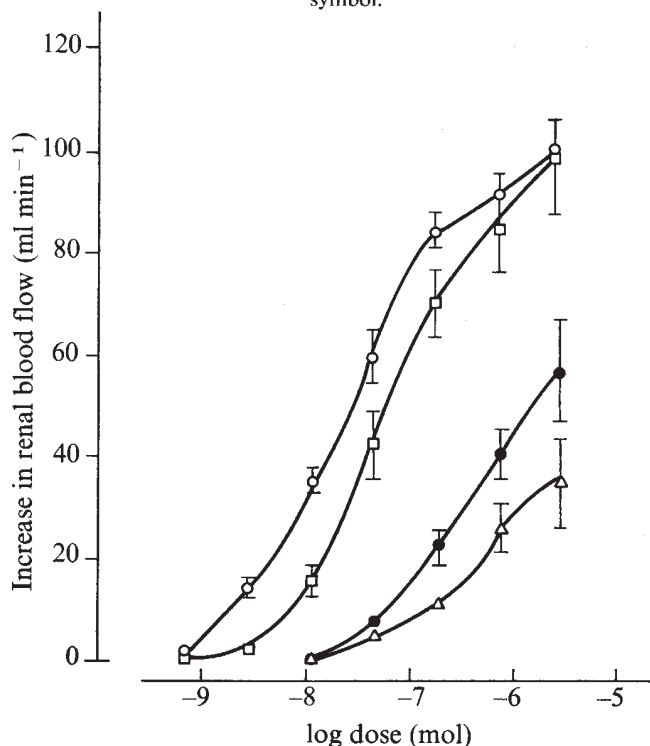


Fig. 1 Mean increments in renal blood flow produced by intra-arterial injection of several doses of: ○, ADTN; □, dopamine; ●, PNA, and △, apomorphine. ADTN was compared with dopamine in five experiments; PNA and apomorphine were compared with dopamine in six more experiments. The dopamine curve represents the mean values obtained in all eleven experiments. Phenoxybenzamine (5 or 10 mg kg⁻¹) was administered in the renal artery before injection of the vasodilators. Vertical lines indicate the s.e.m. when this value was larger than the symbol.

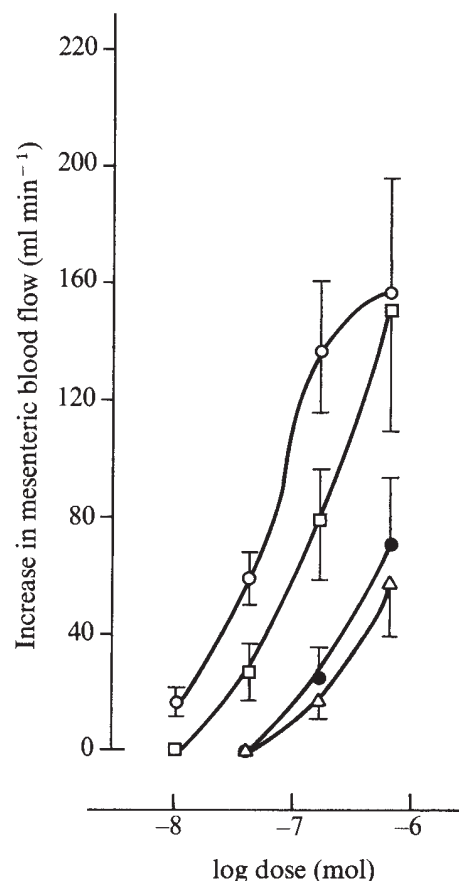


Fig. 2 Mean increments in mesenteric blood flow produced by intra-arterial injections of several doses of: ○, ADTN; □, dopamine; ●, PNA, and △, apomorphine in five experiments in anaesthetised dogs. Phenoxybenzamine (5 or 10 mg kg⁻¹) was administered intra-arterially before injection of vasodilators. Vertical lines indicate the standard error of the mean when this value was greater than the symbol.

agents were administered after intra-arterial injection of 5 or 10 mg of phenoxybenzamine, which was sufficient to eliminate or reverse the vasoconstricting actions of nor-adrenaline. Injections were made in a volume which caused no injection artefacts (0.2–0.4 ml). Dopamine, ADTN, PNA and apomorphine were administered in a fourfold geometric dose progression in doses ranging from 3×10^{-9} to 3×10^{-6} mol. The effects of ADTN and dopamine were investigated in one group of five dogs; in a separate group of six dogs, the effects of PNA were compared with both dopamine and apomorphine. In mesenteric blood flow experiments all agents were investigated in the same five dogs. In femoral blood flow experiments ADTN, dopamine and bradykinin were compared in one group of five dogs and PNA, apomorphine, bradykinin and dopamine in a separate group of five dogs. When large doses of the compounds were injected intra-arterially, recirculation usually had a subsequent effect on blood pressure. Only those changes preceding the alteration in blood pressure were considered in the analysis of the results.

The effects of various antagonists were investigated in two experiments with the renal vascular bed. (1) In five dogs, haloperidol (1.4×10^{-7} mol) was injected into the renal artery simultaneously with the putative agonists. This dose of haloperidol causes transient selective attenuation of the vasodilation produced by dopamine¹². (2) The agonists were administered intra-arterially to four dogs before and after intra-arterial injection of a solution containing propranolol (2 mg kg⁻¹) and atropine (1 mg kg⁻¹): these doses were sufficient to attenuate markedly the vasodilation produced by acetylcholine and isoproterenol. ADTN was synthesised

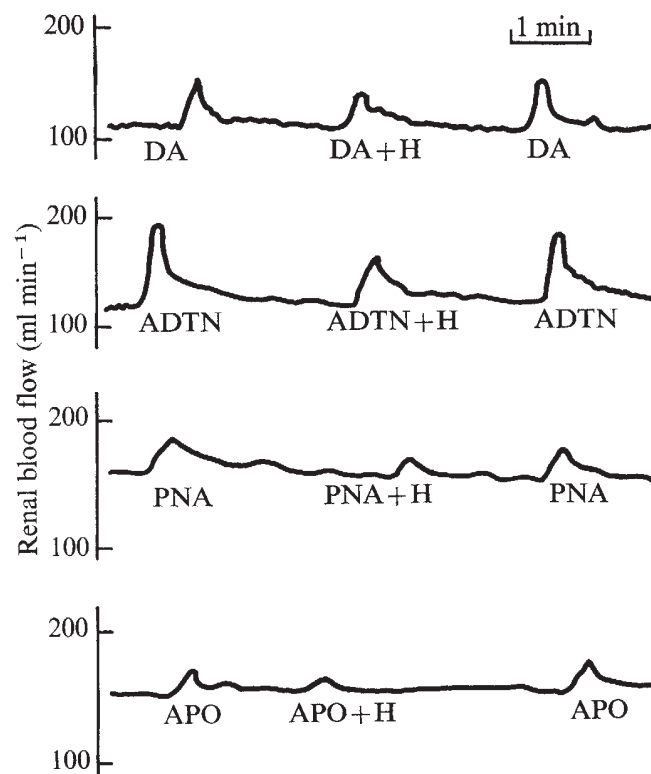


Fig. 3 Effects of injections of dopamine (4.7×10^{-8} mol), ADTN (4.7×10^{-8} mol), PNA (7.5×10^{-7} mol), and apomorphine (7.5×10^{-9} mol) alone and combined with haloperidol (1.4×10^{-7} mol). Phenoxybenzamine (5 or 10 mg kg $^{-1}$) was administered in the renal artery before injection of vasodilators. Dopamine and ADTN were administered in one experiment; PNA and apomorphine in a different experiment.

by R.M.P., PNA by W.B.H. Sources of the other compounds were as follows: dopamine HCl (Calbiochem), noradrenaline bitartrate, isoproterenol hydrochloride, bradykinin and acetylcholine (Sigma), phenoxybenzamine (Smith, Kline and French), propranolol HCl (Ayerst). The paired *t* test was used for statistical analyses.

Dopamine and ADTN increased renal blood flow in a dose-related manner (Fig. 1). In most experiments, equimolar doses of ADTN produced greater vasodilation than dopamine (Fig. 3) and the differences were statistically significant ($P < 0.5$) at three of the doses studied (3×10^{-9} mol, 1.2×10^{-8} mol, 9×10^{-7} mol). PNA and apomorphine were much less potent and effective than ADTN and dopamine. In most experiments, PNA produced greater renal vasodilation than apomorphine and the differences in the mean values were statistically significant for doses of 1.9×10^{-7} mol, 7.5×10^{-7} mol and 3×10^{-6} mol.

Similar responses were produced in mesenteric blood flow (Fig. 2). As in previous studies with dopamine, the dose-response curves were steeper than those constructed from experiments in the renal artery¹². Again, ADTN was more effective than dopamine in most experiments and the difference was statistically significant at two doses, 1.2×10^{-8} mol and 1.9×10^{-7} mol. PNA was a more effective vasodilator than apomorphine in all experiments. But unlike the situation in renal experiments, the mean differences were not statistically significant at any dose.

Arterial blood pressure was not changed appreciably with smaller doses of the vasodilators. But with the highest two doses profound hypotension frequently occurred, making it difficult to estimate the maximum increase in renal and mesenteric blood flow. The hypotension was more pro-

nounced in the mesenteric experiments and thus data from the highest dose are not included in Fig. 2.

In contrast to the vasodilation produced by ADTN, PNA, apomorphine and dopamine in the renal and mesenteric vascular beds, femoral blood flow was not altered by these agents. As in previous studies¹¹, bradykinin in doses ranging from 1×10^{-11} mol to 5×10^{-11} mol increased femoral blood flow in every experiment.

Haloperidol (1.4×10^{-7} mol) caused transient attenuation of the renal vasodilating effects of ADTN, PNA and apomorphine in all experiments and at every dose (Fig. 3).

Combined administration of propranolol and atropine did not affect the renal vasodilation produced by ADTN, PNA or apomorphine. These agents, however, virtually abolished the vasodilation produced by isoproterenol in the dose range 3×10^{-8} mol to 3×10^{-7} mol and acetylcholine in the dose range 5×10^{-7} mol to 3×10^{-6} mol.

These experiments have demonstrated that ADTN, PNA and apomorphine satisfy the criteria established for *in vivo* characterisation of dopamine-like vasodilators^{4,13}. First, after administration of phenoxybenzamine, dose-related vasodilation occurred in the renal and mesenteric vascular beds, but not in the femoral vascular bed. Second, vasodilation was not antagonised by propranolol and atropine but was attenuated selectively by haloperidol. Accordingly, the series of dopamine-like vasodilators has been extended to include more rigid cyclic compounds. In a previous investigation, apomorphine seemed to be dopamine-like, but haloperidol attenuation was not demonstrated⁴.

The greater efficacy of ADTN compared with PNA or apomorphine indicates that the preferred conformation for dopamine-induced vasodilation is the fully extended transoid form. A similar conclusion was reached⁶⁻⁸ for putative dopamine receptors in the CNS. Indeed, the almost identical potency series of agonists causing dopamine-like effects in the blood vessels and in the brain, and the observations that phenothiazines and butyrophenones antagonise both central and vasodilating effects of dopamine support the suggestion that dopamine receptors in the two regions are identical^{1,2,14}.

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HAROLD J. CRUMLEY, JR

Department of Pharmacology,
Emory University School of Medicine,
Atlanta, Georgia 30322

ROGER M. PINDER

Australasian Drug Information Services,
PO Box 34-030,
Birkenhead, Auckland 10,
Australia

W. BANKS HINSHAW

Sterling-Winthrop Research Institute,
Rensselaer, New York 12144

LEON I. GOLDBERG*

Committee on Clinical Pharmacology,
University of Chicago,
Chicago, Illinois 60637

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*To whom reprint requests should be sent.

- Iversen, L. L., *Science*, **188**, 1084-1089 (1975).
- Goldberg, L. I., *Pharmac. Rev.*, **1**, 29 (1972).
- Furchgott, R. F., in *Catecholamines* (edit. by Blaschko, H., and Sandler, M.), 283-335 (Springer, Berlin, 1972).
- Goldberg, L. I., Sonnevill, P. F., and McNay, J. L., *J. Pharmac. exp. Ther.*, **63**, 188-197 (1968).
- Miller, R. J., Horn, A. S., Iversen, L. L., and Pinder, R. M., *Nature*, **250**, 238-241 (1974).
- Woodruff, G. N., Elkhawad, A. O., and Pinder, R. M., *Eur. J. Pharmac.*, **45**, 80-86 (1974).
- Costall, B., Naylor, R. J., and Pinder, R. M., *J. Pharm. Pharmacol.*, **26**, 753-762 (1974).
- Costall, B., Naylor, R. J., and Pinder, R. M., *Eur. J. Pharmacol.*, **31**, 94-109 (1975).
- Cannon, J. B., *Adv. Neurol.*, **9**, 177-183 (1975).
- Koch, M. D., Cannon, J. D., and Burkman, A. M., *J. med. Chem.*, **11**, 977-981 (1968).
- McNay, J. L., Goldberg, L. I., *J. Pharmac. exp. Ther.*, **151**, 23-31 (1966).

¹² Yeh, B. K., McNay, J. L., and Goldberg, L. I., *J. Pharmac. exp. Ther.* **168**, 303-309 (1969).

¹³ Goldberg, L. I., *Biochem. Pharmac.* **24**, 651-653 (1975).

¹⁴ Goldberg, L. I., *Adv. Neurol.*, **9**, 53-56 (1975).

Possible involvement of central histamine H₂-receptors in the hypotensive effect of clonidine

CLONIDINE and some other antihypertensive drugs exert their effect by acting on the central nervous system¹⁻⁴. Some α -adrenoceptor blocking drugs antagonise the centrally mediated hypotensive effect of clonidine⁵⁻⁷. It has therefore been suggested that clonidine acts by stimulating central α -adrenoceptors, bringing about a depression of the cardiovascular system. The involvement of classical α -adrenoceptors has, however, been challenged^{8,9}. Moreover, in the brain tissue clonidine may even be a potent α -adrenoceptor antagonist¹⁰.

We have previously reported that clonidine stimulates histamine H₂-receptors in the gastric mucosa¹¹, and recently it has been shown that clonidine also has the same effect in the heart¹².

Histamine H₂-receptors are present in the brain tissue¹³. Interestingly, alterations in the metabolism of brain histamine have been implicated in the pathogenesis of the spontaneous hypertension in rats¹⁴. We have now used the specific histamine H₂-receptor antagonist, metiamide¹⁵, in order to study whether central histamine H₂-receptors might be involved in the cardiovascular effects of clonidine.

Female Sprague-Dawley rats (200-250 g) were anaesthetised with urethane (1.5 g kg⁻¹ intraperitoneally). The trachea was cannulated with a polyethylene tube and the rats were allowed to breathe spontaneously. The mean blood pressure was measured directly from the left femoral artery by means of a pressure transducer (Harvard apparatus 377). The heart rate was calculated from the pulse waves. Since

Fig. 1 Influence of metiamide on the hypotensive effect of clonidine in rats. Metiamide (1.5 μ mol per rat) or saline was administered intracerebroventricularly, 10-15 min later clonidine (30 μ g kg⁻¹) was injected intravenously. Differences between the metiamide (●) and control (○) groups were significant to the $P < 0.001$ level for all measurements except that made at 40 min ($P < 0.02$). The time elapsed after the injection of clonidine is shown on the abscissa. Vertical bars indicate s.e.m. The number of rats was 18 in the control group and 10 in the metiamide group.

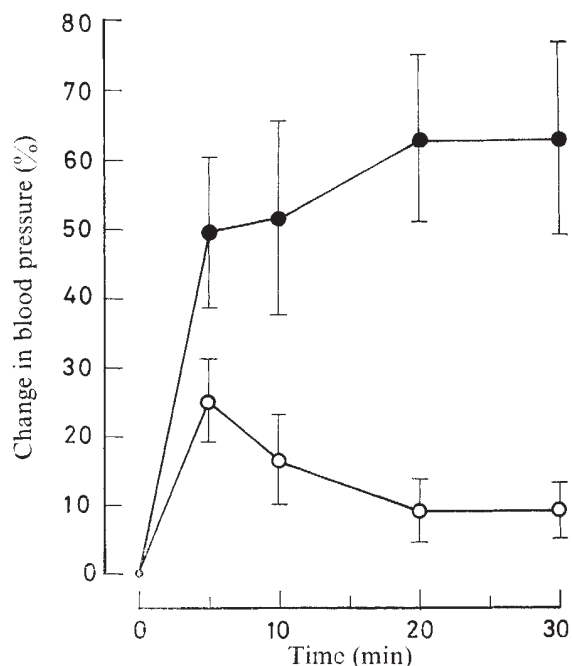
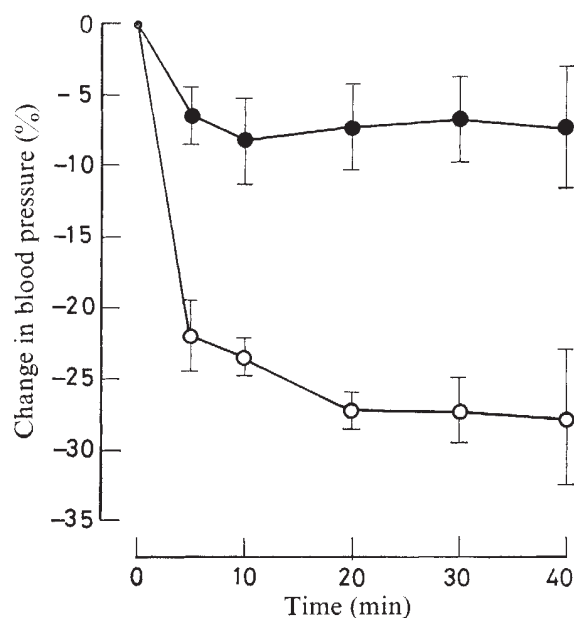


Fig. 2 Reversal of the clonidine-induced hypotension by metiamide. Clonidine (30 μ g kg⁻¹ intravenously) induced hypotension as shown in Fig. 1. 30 min after the injection of clonidine, metiamide (1.5 μ mol per rat) or saline was administered intracerebroventricularly. The time elapsed thereafter is shown on the abscissa. The differences between the metiamide (●) and control (○) groups were statistically significant at the $P < 0.005$ level after 20 min, and the $P < 0.01$ level after 30 min. Vertical bars, s.e.m. The number of rats was 9 in the control group and 6 in the metiamide group.

metiamide does not penetrate the blood-brain barrier, it was administered intracerebroventricularly.

In the first series of experiments, metiamide (1.5 μ mol per rat) was infused during 15 min into the lateral cerebral ventricle in a volume of 45 μ l (pH 6). Ten to fifteen minutes after the completion of the metiamide infusion, 30 μ g kg⁻¹ of clonidine hydrochloride was injected into the left femoral vein. The control rats were treated in exactly the same manner but received saline (pH 6) instead of metiamide solution.

The blood pressure values before the clonidine injections were 110 ± 4 (s.e.) mmHg in the control rats and 109 ± 5 (s.e.) mmHg in the metiamide-pretreated rats.

In the control rats clonidine induced a marked hypotension which was obvious for at least 40 min (Fig. 1). The maximum fall of blood pressure (approximately 30% from the initial level) was reached within 20 min and the blood pressure remained nearly constant for at least another 20 min. Metiamide alone did not affect the blood pressure. In the metiamide-pretreated rats, however, the hypotensive response to clonidine was markedly attenuated (Fig. 1) and the blood pressure remained for the whole 40-min observation period significantly higher ($P < 0.02$ to $P < 0.001$) than in the control rats. The bradycardiac effect of clonidine was slightly but not significantly ($P > 0.05$) antagonised by the metiamide pretreatment.

In another series of experiments the ability of intracerebroventricular metiamide to reverse the clonidine-induced hypotension was studied. Thirty minutes after the intravenous injection of clonidine (30 μ g kg⁻¹), 1.5 μ mol of metiamide was injected intracerebroventricularly within 2 min in a volume of 15 μ l. The control rats received 15 μ l of saline. Metiamide caused a clear elevation of the blood pressure level within 5 min (Fig. 2). The metiamide-induced increase of the heart rate was not statistically significant ($P > 0.05$).

In contrast to metiamide, the histamine H₁-receptor

blocking agent diphenhydramine (0.03 to 1.5 μmol per rat intracerebroventricularly) did not antagonise the hypotensive effect of clonidine. The highest dose (1.5 μmol per rat) was lethal to most of the rats.

The present results indicate that clonidine also stimulates histamine H_2 -receptors in the central nervous system as it does in peripheral tissues^{11,12}. Moreover, the findings also suggest that a stimulation of central histamine H_2 -receptors brings about a depression of the cardiovascular system which is manifested as the lowering of blood pressure.

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HEIKKI KARPPANEN
ILARI PAAKKARI
PIRKKO PAAKKARI
RAIMO HUOTARI
ANNA-LIISA ORMA

Department of Pharmacology,
University of Oulu,
SF-90220 Oulu 22, Finland

Received November 20; accepted December 14, 1975.

- ¹ Kobinger, W., and Walland, A., *Eur. J. Pharmac.*, **2**, 155 (1967).
- ² Sattler, R. W., and van Zwieten, P. A., *Eur. J. Pharmac.*, **2**, 9 (1967).
- ³ Schmitt, H., Schmitt, H., Boissier, J. R., and Guidicelli, J. F., *Eur. J. Pharmac.*, **2**, 147 (1967).
- ⁴ van Zwieten, P. A., *J. Pharm. Pharmac.*, **25**, 89 (1973).
- ⁵ Schmitt, H., Schmitt, H., and Fenard, S., *Eur. J. Pharmac.*, **14**, 98 (1971).
- ⁶ Haessler, G., *Naunyn-Schmiedeberg's Archs Pharmac.*, **278**, 231 (1973).
- ⁷ Finch, L., Buckingham, R. E., Moore, R. A., and Bucher, T. J., *J. Pharm. Pharmac.*, **27**, 181 (1975).
- ⁸ Schmitt, H., Schmitt, H., and Fenard, S., *Arzneimittel-Forschung*, **11**, 625 (1973).
- ⁹ Bolme, P., Corrodi, H., Fuxe, K., Hökfelt, T., Lidbrink, P., and Goldstein, M., *Eur. J. Pharmac.*, **28**, 89 (1974).
- ¹⁰ Skolnick, P., and Daly, J. W., *J. Pharmac. exp. Ther.*, **193**, 549 (1975).
- ¹¹ Karppanen, H. O., and Westermann, E., *Naunyn-Schmiedeberg's Archs Pharmac.*, **279**, 83 (1973).
- ¹² Csongrady, A., and Kobinger, W., *Naunyn-Schmiedeberg's Archs Pharmac.*, **282**, 123 (1974).
- ¹³ Baudry, M., Martres, M.-P., and Schwartz, J.-Ch., *Nature*, **253**, 362 (1975).
- ¹⁴ Spector, S., Tarver, J., and Berkowitz, B., in *Spontaneous Hypertension* (edit. by Okamoto, K.), 44 (Igaku Shoin, Tokyo, 1972).
- ¹⁵ Black, J. W., Duncan, W. A. M., Emmett, J. C., Ganellin, C. R., Hesselbo, T., Parsons, M. E., and Wyllie, J. H., *Agents and Actions*, **3**, 133 (1973).

Induction of ovalbumin and conalbumin synthesis in immature chick oviducts by ethionine

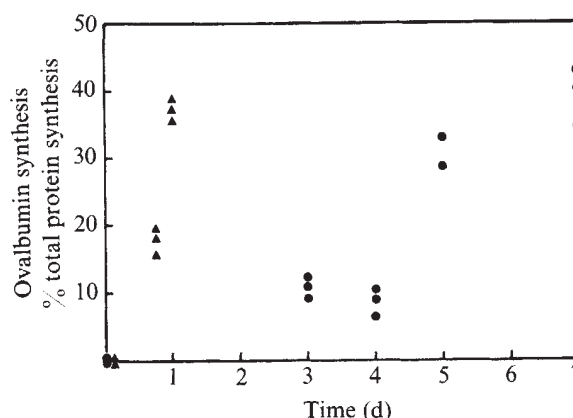
PROLONGED feeding of rats with ethionine results in a high frequency of hepatic carcinoma. Ethionine induces other pathological manifestations in other organs and tissues of experimental animals¹, and some of its effects can be reversed rapidly by the administration of methionine. At the molecular level ethionine induces a rapid decrease in hepatic ATP concentration²⁻⁴, followed by inhibition of RNA⁵ and protein synthesis⁶. The decrease in ATP concentration is due to formation of S-adenosyl-ethionine⁷, an inhibitor of tRNA methyltransferases^{8,9}, and so tRNA isolated from the liver of rats injected with adenine and ethionine is hypomethylated^{10,11}. It is well established that several eukaryotic mRNAs are methylated on the 5' terminus and that such modification is essential for translation^{12,13}. To study the mechanism of methylation we attempted to produce a specific methyl-deficient mRNA by the administration of ethionine. To monitor the effectiveness of the deprivation of methyl groups the synthesis of hormone-induced ovalbumin in immature chick oviducts was followed. Administration of oestrogen to immature chicks causes cytodifferentiation and growth of the primitive oviduct¹⁴⁻¹⁷. The tubular gland cells synthesise ovalbumin, conalbumin, ovomucoid and lysozyme which comprise 85-90% of the egg-white proteins¹⁸. The con-

tinuous presence of oestrogen is required for sustained synthesis of these proteins in immature chicks; withdrawal of oestrogen is accompanied by a gradual decline in cell-specific protein synthesis as well as in the weight of the oviduct and the RNA content of the tissue¹⁷. But, the tubular gland cells in the oviduct magnum during withdrawal are retained although they do not synthesise cell-specific secretory proteins^{17,19}. Readministration of hormone to chicks after withdrawal (secondary stimulation) results in restoration of cell-specific protein synthesis without concomitant need for DNA synthesis¹⁷. During these studies ethionine unexpectedly simulated the effects of the injection of oestrogen for secondary stimulation.

Four-day-old Calhoun chicks were injected with 1 mg of oestradiol benzoate in sesame oil for 10 d (ref 19). After a withdrawal period of 3-4 weeks the chicks were used in most of the experiments. The chicks were injected in groups of eight intraperitoneally with a 1-ml suspension in water of L-ethionine or methionine (0.25 mg per g body weight) and/or adenine (0.12 mg per g body weight) for 5-7 d. At the times specified the animals were killed and the capacity for ovalbumin synthesis of the magnum explants was determined. Oestradiol benzoate-17 β was a product of Calbiochem, L-ethionine, adenine and methionine were from Sigma. ¹⁴C-amino acid mixture (algal profile) was purchased from Schwarz/Mann. Medium 199 ($\times 10$) and NaHCO₃-free Hanks balanced salt solution ($\times 10$) were from Grand Island Biological. Anti-ovalbumin and conalbumin sera were elicited in rabbits.

Chicks were decapitated and their oviducts were removed aseptically, the magnum portions of the oviduct were freed of adjoining tissue and were cut into small pieces. The magnum explants were incubated *in vitro* in antibiotic-free medium²⁰⁻²². Tissue (100-200 mg) was incubated in 2 ml of medium in 25-ml, rubber stoppered Erlenmeyer flasks. Each flask contained tissue from at least three oviduct magna. The incubations were carried out at 37 °C for 3 h with constant shaking and the flasks were gassed with 95% O₂-5% CO₂ at hourly intervals. At the end of the incubation the pieces of tissue were blotted on filter paper, homogenised in 2 ml of 15 mM NaCl-10 mM sodium phosphate, pH 7.5, and centrifuged at 100,000g for 1 h. The high speed supernatant was used for subsequent analyses²⁰. Labelled ovalbumin was determined essentially according to Rhoads *et al.*²³. The tubes were incubated at 37 °C for 1 h and then stored at 4 °C until they were filtered. The precipitates were collected on Millipore HAWP filters, 0.45 μm (pre-wetted with the wash solution) and were washed three times

Fig. 1 Time course of induction of ovalbumin synthesis in immature chick oviducts by oestradiol ($\blacktriangle$), and ethionine and adenine ($\bullet$). Chicks withdrawn from primary stimulation for 4 weeks were injected with oestradiol (1 mg) or ethionine and adenine as described in the text. At various times five chicks were killed and ovalbumin synthesis was determined in duplicate or triplicate samples. Details are the same as for Table 1.



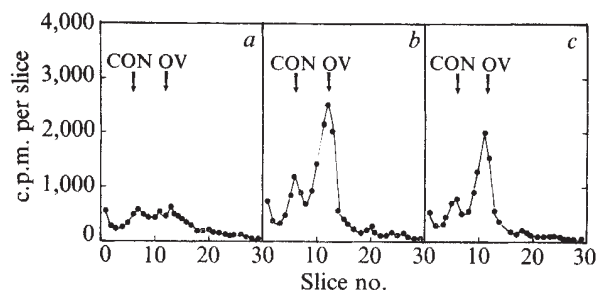


Fig. 2 SDS-acrylamide gel electrophoresis of high speed supernatants from incubations. Oviduct explants were incubated as described in the text. Samples of 50–100 μ l of the high speed supernatant were precipitated with trichloroacetic acid and electrophoresed on SDS-acrylamide gels²⁰. The migration of conalbumin (CON) and ovalbumin (OV) is indicated. *a*, Chicks withdrawn for 28 d, after 10 d of primary oestrogen stimulation; *b*, withdrawn chicks injected with oestrogen for 24 h; *c*, withdrawn chicks injected with ethionine and adenine for 5 d.

with 1-ml samples of 10 mM sodium phosphate, pH 7.5 containing 150 mM NaCl, 1% deoxycholate and 1% Triton X-100. The filters were dried at 100 °C for 30 min and counted in a liquid scintillation counter. The reactions were performed so that at least three concentrations of ovalbumin precipitated in the linear range (antibody excess). A blank of bovine serum albumin (5 μ g) and rabbit antiovine serum albumin was used to correct for non-specific precipitation²⁴.

When, 4 weeks after primary oestrogen stimulation—at which time oviducts do not synthesise ovalbumin—ethionine and adenine were injected for 3 d followed by secondary oestrogen stimulation with oestradiol for 18 h, the outcome was unexpected. Ovalbumin synthesis in these chicks was greater than in those which received oestradiol alone (data not shown). Therefore, the effect of the administration of ethionine without secondary oestrogen stimulation was studied in chicks 4 weeks after primary stimulation (Table 1).

Ethionine itself induced ovalbumin synthesis although a combination of ethionine and adenine was more effective. Adenine alone and in combination with methionine did not induce ovalbumin synthesis. The induction of ovalbumin synthesis by ethionine was slower than by oestrogen (Fig. 1). Ethionine is highly toxic to chicks and the animals died after 5–7 d. Conalbumin synthesis was also induced by ethionine as measured by precipitation with monospecific antiserum to conalbumin (data not shown). Induction of ovalbumin and conalbumin synthesis was confirmed by sodium dodecyl sulphate (SDS)-acrylamide gel electrophoresis (Fig. 2).

Electron microscopy of the oviduct revealed in the control tissues well developed tubular glands but the cytoplasm of the glandular cells was devoid of the organelles associated with specific intracellular synthesis (Fig. 3*a*). In the tissues of ethionine-treated chicks the nuclei of the glandular cells were located basally, the rough endoplasmic reticulum and the Golgi complex were well developed, electron-dense secretory granules were prominent and the

Fig. 3 Electron micrographs of chick oviduct magna. The excised oviduct magnum tissue was minced into 1 mm cubes, fixed in buffered 4% glutaraldehyde for 1 h, postfixed in 1% osmium tetroxide, dehydrated in ethanol and embedded in Epon-Araldite via propylene oxide. Thin sections were stained with hot alcoholic uranyl acetate²⁵ and lead citrate²⁶. *a*, Tubular gland from control chicks, the nuclei are randomly distributed and the cytoplasm has a minimal number of organelles ($\times 6,250$). *b*, Tubular gland from ethionine-treated chicks; the nuclei are basal; the cytoplasm contains abundant rough endoplasmic reticulum, Golgi complex and apical-secretory granules ($\times 6,500$). *c*, Tubular gland from oestradiol-treated chicks, the cytoplasmic organelles are even more prominent and there are abundant ribosomes ($\times 6,750$).

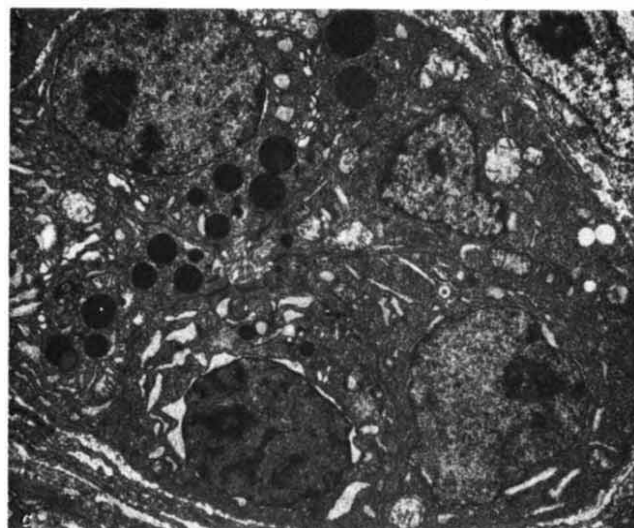
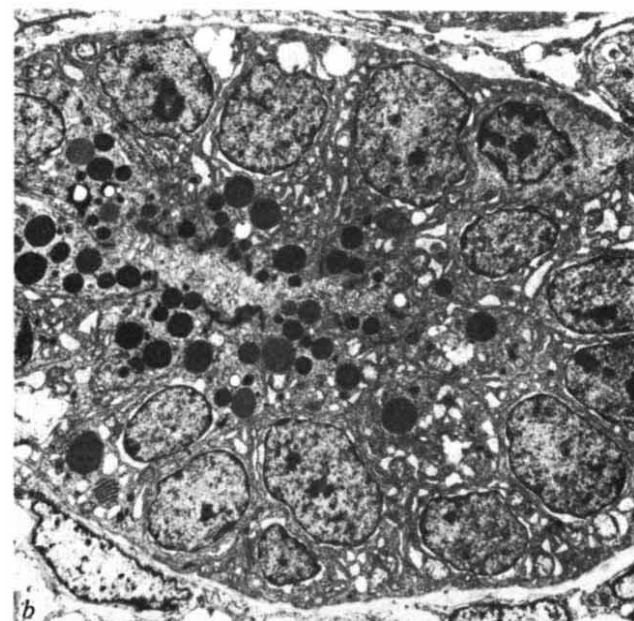
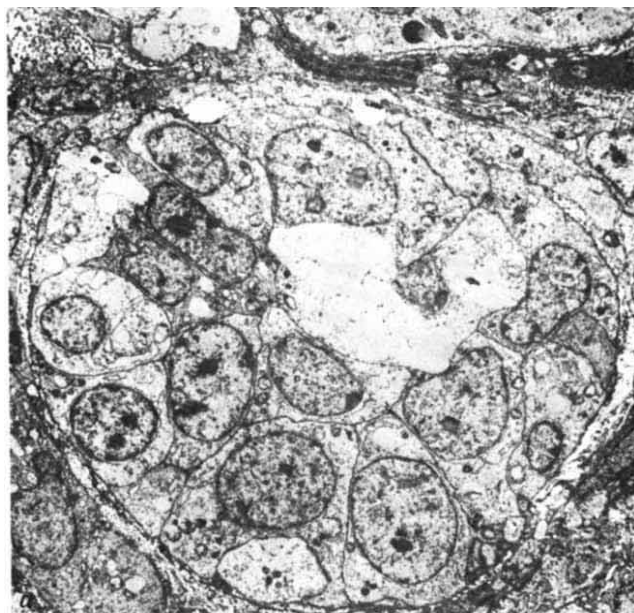


Table 1 Induction of ovalbumin synthesis in immature chick oviducts by ethionine

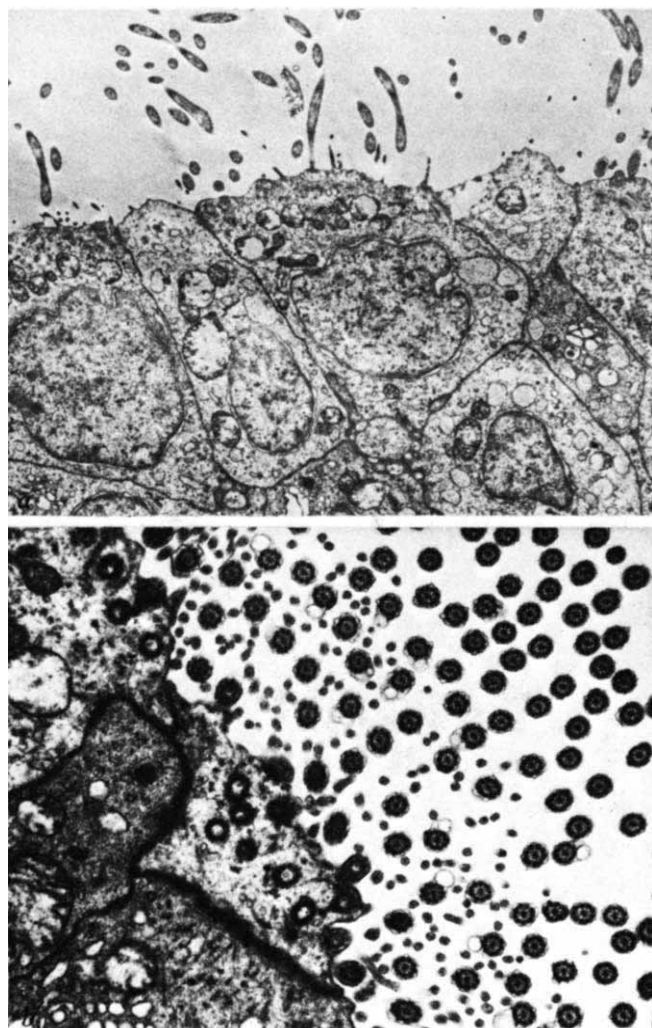
Treatment	Oviduct magnum* weight per chick (mg)	¹⁴ C-amino acids incorporated per mg of soluble protein (c.p.m. $\times 10^{-5}$)	¹⁴ C-amino acids incorporated into ovalbumin per mg of soluble protein (c.p.m. $\times 10^{-5}$)	Ovalbumin synthesis, % of total protein synthesis
None	230 (4)	0.77 0.85	Not detected	
Oestradiol†	580 (4)	0.83 0.98	0.354 0.369	42.7 37.7
Ethionine	260 (8)	1.53 1.14	0.384 0.108	25.1 9.5
Ethionine+ adenine	300 (7)	1.42 1.23	0.498 0.598	35.1 48.6
Adenine	180 (8)	1.10 1.01	Not detected	
Adenine+ methionine	230 (8)	0.77 0.85	Not detected	

After 10 d of primary oestrogen stimulation chicks were withdrawn for 28 d and in groups of eight were injected intraperitoneally for 5 d as described in the text. On day 6 the animals were killed and ovalbumin synthesis in the oviduct magnum explants was determined as described. The incubations were carried out in duplicate and the flasks contained tissue from at least two different chicks.

*Average magnum weight, the number in parentheses refers to the number of chicks.

†Secondary oestrogen stimulation was by 1 mg of oestradiol for 24 h.

Fig. 4 Electron micrographs of epithelia of chick oviduct magna. Tissue processing was identical to that described for Fig. 3. Surface epithelium from control chicks (*a*), the epithelial cells contain sparse cytoplasmic organelles, few cilia are present in the surface ($\times 5,000$). *b*, Surface epithelium from ethionine-treated chicks. The cells contain abundant cytoplasmic organelles and cilia are prominent in the oviduct cavity ($\times 6,500$).



cells had membrane specialisation resembling tight junctions, desmosomes and microvilli (Fig. 3*b*). In the cells of oestradiol-treated oviduct, the nuclei were basal, and the rough endoplasmic reticulum and Golgi complexes were even better developed than in the tissue of ethionine-treated animals. The secretory granules were more prominent, membrane differentiation was obvious and the cytoplasm contained abundant ribosomes (Fig. 3*c*). A process similar to that in the glands, was obvious in the surface epithelium^{27,28}. The surface cells in the controls had few organelles, only occasional cilia and sparse membrane specialisation. In the tissues of the animals exposed to oestradiol or ethionine, cell organelles, membrane specialisation and cilia became abundant (Fig. 4).

Induction of ovalbumin and conalbumin synthesis by ethionine was not observed in the "primitive oviduct" of chicks (5–6 weeks old) which had not received primary stimulation with oestrogen. This suggests that ethionine alone cannot cause cytodifferentiation and growth of the primitive oviduct, but that in tissues already differentiated by the action of oestradiol it can induce the further cellular differentiation needed for specific protein synthesis. The mechanism of induction of protein synthesis by ethionine remains obscure: it may or may not stem from interference with the methylation of either mRNA or tRNA or both. In view of the powerful oncogenicity of the agent the phenomenon is, however, worthy of study to delineate the mechanism of its induction.

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OPENDRA K. SHARMA

ERNEST BOREK

Department of Microbiology

ANTONIO MARTINEZ-HERNANDEZ

Department of Pathology,
University of Colorado Medical Center,
Denver, Colorado 80220

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¹ Farber, E., *Adv. Cancer Res.*, **7**, 383–474 (1963).

² Farber, E., Shull, K. H., Villa-Trevino, S., Lombardi, B., and Thomas, M., *Nature*, **203**, 34–40 (1964).

³ Shull, K. H., *J. biol. Chem.*, **237**, 1734–1735 (1962).

⁴ Villa-Trevino, S., Shull, K. H., and Farber, E., *J. biol. Chem.*, **238**, 1757–1763 (1963).

⁵ Villa-Trevino, S., Shull, K. H., and Farber, E., *J. biol. Chem.*, **241**, 4670–4674 (1966).

- 6 Farber, E., and Corban, M. S., *J. biol. Chem.*, **233**, 625-630 (1958).
- 7 Shull, K. H., McConomy, J., Vogt, M., Castillo, A., and Farber, E., *J. biol. Chem.*, **241**, 5060-5070 (1966).
- 8 Moore, B. G., and Smith, R. C., *Can. J. Biochem.*, **47**, 561-565 (1969).
- 9 Moore, B. G., *Can. J. Biochem.*, **48**, 702-705 (1970).
- 10 Rajalakshmi, S., *Proc. Am. Ass. Cancer Res.*, **14**, 39 Abstr. (1973).
- 11 Kerr, S. J., *Cancer Res.*, **35**, 2969-2973 (1975).
- 12 Rottman, F., Shatkin, A. J., and Perry, R. P., *Cell*, **3**, 197-199 (1974).
- 13 Muthukrishnan, S., Both, G. W., Furuichi, Y., and Shatkin, A. J., *Nature*, **255**, 33-37 (1975).
- 14 O'Malley, B. W., McGuire, W. L., Kohler, P. O., and Korenman, S. G., *Rec. Prog. Hormone Res.*, **25**, 105-160 (1969).
- 15 Kohler, P. O., Grimley, P. M., and O'Malley, B. W., *J. Cell Biol.*, **40**, 8-26 (1969).
- 16 Oka, T., and Schimke, R. T., *J. Cell Biol.*, **41**, 816-823 (1969).
- 17 Oka, T., and Schimke, R. T., *J. Cell Biol.*, **43**, 123-137 (1969).
- 18 Palmiter, R. D., *J. biol. Chem.*, **247**, 6450-6461 (1972).
- 19 Palmiter, R. D., Christensen, A. K., and Schimke, R. T., *J. biol. Chem.*, **245**, 833-845 (1970).
- 20 Palmiter, R. D., Oka, T., and Schimke, R. T., *J. biol. Chem.*, **246**, 724-737 (1971).
- 21 Sharma, O. K., Mays, L. L., and Borek, E., *J. biol. Chem.*, **248**, 7622-7624 (1973).
- 22 Sharma, O. K., Mays, L. L., and Borek, E., *Biochemistry*, **14**, 509-514 (1975).
- 23 Rhoads, R. E., McKnight, G. S., and Schimke, R. T., *J. biol. Chem.*, **246**, 7407-7410 (1971).
- 24 Sharma, O. K., Roberts, W. K., Beezley, D., and Borek, E., *Biochim. biophys. Acta*, **390**, 327-331 (1975).
- 25 Locke, M., and Krishnan, J., *Cell Biol.*, **50**, 550-553 (1971).
- 26 Reynolds, E. S., *J. Cell Biol.*, **17**, 208-212 (1963).
- 27 Aitken, R. N. C., and Johnston, H. S., *J. Anat.*, **97**, 87-89 (1963).
- 28 Hendler, R. W., *J. biophys. biochem. Cytol.*, **3**, 325-330 (1957).

Regulation of degradation of thyrotropin releasing hormone by thyroid hormones

WHEN thyrotropin releasing hormone (TRH, pyroGlu-His-Pro-NH₂) was isolated and its structure determined^{1,2} the concept of neurohumoral regulation of hormone secretion by the anterior pituitary³ was confirmed, but the mechanisms involved are not well understood. Enzymatic inactivation of TRH by blood and serum may regulate the hormone's activity by determining the number of molecules available to the thyrotroph⁴. With this physiological function, degradative enzyme(s) would itself need to be regulated and I report here that the activity of the TRH-degrading enzyme(s) is controlled by the thyroid hormones.

Rapid inactivation of TRH by serum was reported before its structure was elucidated^{5,6}, but the mechanism of degradation remains to be elucidated. Based on the observation that the free amino acids are the main degradation products⁷, a quantitative test of TRH-degradation was developed. After incubation of TRH (labelled with ³H in the histidine moiety) with serum, the labelled products

could be separated by ion exchange chromatography as described in the legend of Fig. 1. TRH migrated with an *R_f* of 0.25 as identified by cochromatography of synthetic material which was visualised using Pauly reagent. All labelled degradation products remained absorbed near the origin, as shown by rechromatography of the material from the TRH zone with standard peptides. Thin-layer chromatography in several solvent systems and electrophoresis at pH 1.9 and 6.4, revealed one radioactive spot comigrating with the standard hormone while all the possible labelled cleavage products migrated separately (Table 1). Radioactivity in the TRH zone was determined by scintillation counting.

This test provided a simple and sensitive method for quantitative evaluation of TRH degradation by serum (see legend to Fig. 2). It is thus possible to follow the kinetics of TRH degradation by serum and to determine the initial rate as a measure of enzyme activity. In the conditions described (Fig. 2), the initial rate of degradation increased from 1.6% min⁻¹ for serum of untreated rats to 3.3% min⁻¹ for that of hyperthyroid animals (injected with triiodothyronine, T₃). With serum from hypothyroid rats (treated with propylthiouracil, PTU) degradation was very slow (0.6% min⁻¹) but restored to above normal (1.7% min⁻¹) within 72 h by daily injection of 10 µg T₃. This observation parallels the reported reduced TRH inactivation of plasma from hypophysectomised-thyroidectomised rats which was restored by treatment with T₃ (ref. 8). Because of the limitations of the biological assay, these studies could not be confirmed⁸ and have not been extended.

With the demonstration of a single exponential relationship of TRH degradation with time (Fig. 2) and serum protein (our unpublished results) degradation activity could be determined by a four-point assay with four serum dilutions. When the degradation activity of serum from untreated control animals was compared with that from animals injected with T₃ or treated with PTU, the effect of the thyroid hormones could be demonstrated clearly (Fig. 3). As Fig. 3a shows, the activity of the serum enzyme(s) declined slowly when the formation of thyroid hormones

Fig. 1 Separation of labelled split products from TRH-³H-histidine incubated with serum. Trunk blood from rats was collected in plastic tubes and allowed to clot for 3 h at 0 °C. Serum was obtained by centrifugation at 5,000 r.p.m. for 15 min. Serum (10 µl) diluted 1:3 with buffer A (100 mM Tris-HCl pH 7.6, 1 mM DTT), was incubated at 37 °C for 30 min with 1.0 µCi of purified TRH-³H-histidine (18 Ci mmol⁻¹) in 20 µl buffer A. The reaction mixture and 10 µg synthetic TRH were applied to prewashed cellulose phosphate paper which was then developed with 1 N acetic acid. The labelled products were localised by scanning for radioactivity (Berthold Scanner, LB 2723) and the cochromatographed TRH was stained with the Pauly reagent.

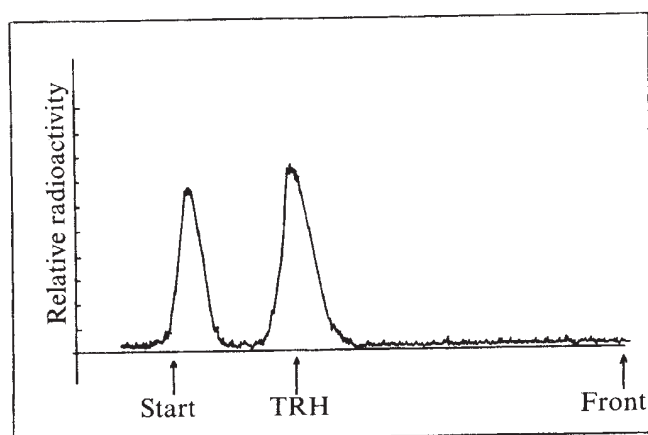


Table 1 Chromatographic separation of TRH from cleavage products

Thin-layer chromatography in solvent system	<i>R_f</i> of substance					
	1	2	3	4	5	6
A	0.57	0.36	0.21	0.58	0.30	0.14
B	0.63	0.5	0.46	0.40	0.27	0.21
C	0.56	0.48	0.37	0.43	0.35	0.19
Paper electrophoresis at	Migration (cm)					
	pH 1.9	pH 6.4	pH 1.9	pH 6.4	pH 1.9	pH 6.4
pH 1.9	14.5	13.5	14.5	30	24.5	24.5
pH 6.4	20.5	6.4	6.5	32	21.5	25.5

The material from the TRH corresponding zone of Fig. 1 was eluted with 0.5 ml 1 N ammonia using extraction thimbles (Reeve Angel) and the standard material was applied to silica gel precoated plates (Merck No. 5721) for thin-layer chromatography or to paper (Whatman 3M) for electrophoresis. 1, TRH; 2, deamido-TRH; 3, pyroGlu-His; 4, His-Pro-NH₂; 5, His-Pro; 6, His. The plates were developed in vapour-saturated tanks using the solvent systems: A, CHCl₃-CH₃OH-NH₃aqueous (125:75:25); B, propanol-methyl-ethylketone-pyridine-acetic acid-water (40:40:40:20:40); C, phenol-water (150:50). Paper electrophoresis was carried out at 50 V cm⁻¹ using buffer pH 1.9 (HCOOH-CH₃COOH-H₂O 50:150:800) or buffer pH 6.4 (pyridine-acetic acid-water 100:10:890). The labelled material was localised by scanning for radioactivity and the cochromatographed standard material by staining with Pauly reagent or by the chlorine-iodide reaction for the phenol-containing solvent system.

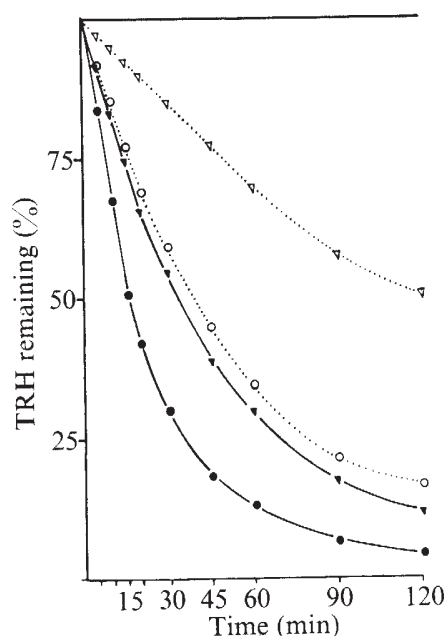


Fig. 2 Time course of degradation of TRH by serum. 0.5 μ Ci of TRH- 3 H-histidine in 40 μ l of buffer was incubated at 37 $^{\circ}$ C with 20 μ l of serum (diluted 1:3 with buffer A). At various times 5 μ l of the reaction mixture was spotted on the ion exchange paper. After development in 1 N acetic acid the strips were segmented and the segments corresponding to the TRH zones were placed in scintillation vials. 500 μ l 2 N ammonia per segment (1.5×2 cm) was added to each vial and after 10 min 10 ml of Bray solution was added before scintillation counting. The values are expressed as percentage of the total TRH added. The serum was obtained, as for Fig. 1, from one group of male rats, 6 months old. $\circ$, Untreated; $\bullet$, 10 μ g T_3 per 100 g body weight given subcutaneously every 24 h, 72 h before decapitation; ∇ , PTU (200 mg l^{-1}) was added to the drinking water for 4 months; $\blacktriangledown$, PTU-pretreated rats were injected with T_3 as before.

was blocked by the goitrogenic agent PTU. In the conditions described (Fig. 3) peptidase activity was reduced by 50% after 40 d. Whether the slow decline reflects the long half life of the circulating enzyme(s) needs further investigation.

In contrast, a rapid increase of enzymatic activity became evident 24 h after injection of normal and hypothyroid animals with T_3 (Fig. 3b): 72 h after injection there had been a 2.1-fold increase of enzymatic activity for euthyroid animals. The rate of increase was greater for euthyroid animals than for those pretreated with PTU. In parallel with biological activity, the stimulation of the enzyme activity was less pronounced when thyroxine (T_4 , Sigma) was injected, but the onset of hormone expression was the same. The prolonged lag period and the preliminary observation that stimulation is inhibited by actinomycin D or puromycin indicate that the appearance of enzymatic activity is preceded by the induction of protein (enzyme) synthesis, as with the induction of hepatic cellular activities by thyroid hormones⁹ or feedback control of pituitary thyroid stimulating hormone (TSH) secretion by T_3 or T_4 (refs 10–12).

As Fig. 3c shows, the effect of the thyroid hormones is strongly dose related. Even after subcutaneous injection of only 0.5 μ g T_3 per 100 g body weight per day, there was strong stimulation of enzymatic activity. Euthyroid female rats showed only 85% of the enzymatic activity of euthyroid males. The origin of this sex difference is as obscure as the sex difference of plasma TSH and T_4 in rats¹³. The response to T_3 was less for female and PTU-treated rats than for control males.

These results show that the activity of the TRH-

degrading enzyme(s) is controlled by the thyroid hormones. Because there is evidence that blood collected from the hypothalamo-hypophyseal portal vessels inactivates TRH (ref. 6 and R. L. Eskay, personal communication), my results suggest that the regulation of the positive stimulus of TRH on the thyrotroph could be the physiological function of these enzyme(s). The enzymatic degradation would thus contribute to the balance of thyrotropin secretion, which in turn is controlled by the thyroid hormone-induced negative feedback mechanism acting directly on the thyrotroph^{10–12}. This sensitive and rapid assay provides a tool for further studies of the implication of the enzymatic degradation of TRH in the regulation of the hypothalamic-pituitary-thyroid axis, in clinical diagnosis (TRH-induced TSH-secretion tests) and in pathological disorders (enzyme defects) which might contribute to the "inappropriate secretion of TSH".

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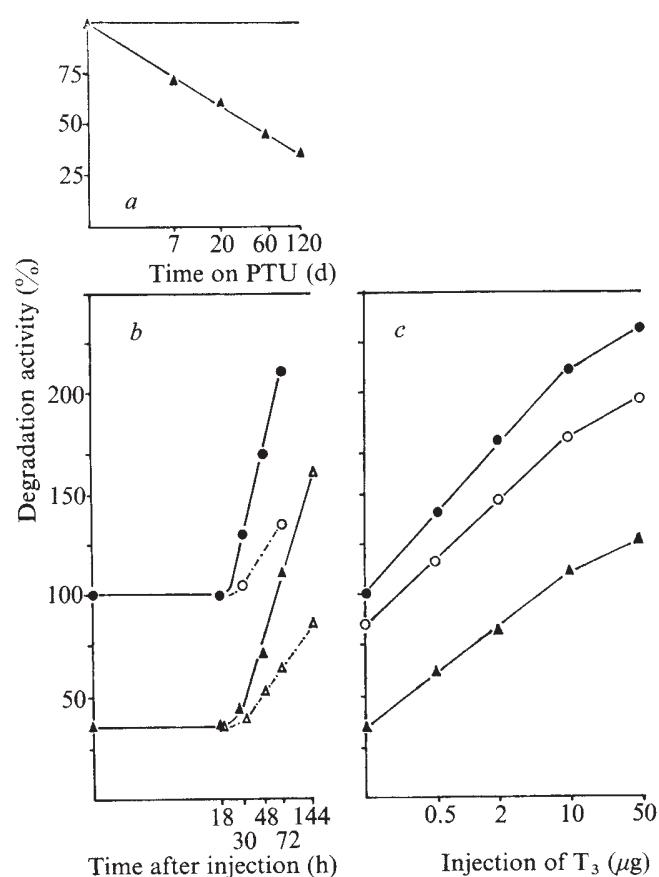


Fig. 3 Effect of thyroid hormones on the degradation of TRH activity of serum. Serum was obtained as for Fig. 1 and assayed at four dilutions (1:1, 1:2, 1:3 and 1:4) following a four-point kinetic (0, 7.5, 15 and 30 min), as described in Fig. 2. Degradation activity is expressed as percentage of the enzyme activity of serum from untreated male rats kept as control animals. **a** TRH degradation activity of serum from male rats after treatment with PTU (200 mg l^{-1} drinking water) for various times. **b**, At given times serum was obtained from 6-month-old male rats, injected subcutaneously with 10 μ g T_3 or T_4 per 100 g body weight every 24 h. $\bullet$, Untreated, injected with T_3 ; $\circ$, untreated injected with T_4 ; $\blacktriangle$, pretreated with PTU (200 mg l^{-1} drinking water) for 4 months, injected with T_3 ; $\triangle$, PTU-pretreated, injected with T_4 . **c**, TRH degradation activity of serum from 6-month-old rats injected subcutaneously every 24 h with the indicated dose of T_3 per 100 g body weight 72 h before decapitation. $\bullet$, Males; $\circ$, females; $\blacktriangle$, males pretreated with PTU as before for 4 months.

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K. BAUER

Max Volmer Institut,
Abteilung Biochemie,
Technische Universität Berlin,
1 Berlin 10, FRG

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- ¹ Burgus, R., Dunn, T. F., Desiderio, D., and Guillemin, R., *C. r. hebdomadaire Séances Acad. Sci., Paris*, **D289**, 1870–1873 (1969).
- ² Bolter, J., Enzmann, F., Folkers, K., Bowers, C. Y., and Schally, A. V., *Biochem. biophys. Res. Commun.*, **37**, 705–710 (1969).
- ³ Harris, G. W., *Neural Control of the Pituitary Gland* (Arnold, London, 1955).
- ⁴ Burgus, R., et al., *Nature*, **226**, 321–325 (1969).
- ⁵ Redding, T. W., and Schally, A. V., *Proc. Soc. exp. Biol. Med.*, **131**, 415–420 (1969).
- ⁶ Vale, W., Burgus, R., Dunn, T. F., and Guillemin, R., *Hormones*, **2**, 193–203 (1971).
- ⁷ Bauer, K., Sy, J., and Lipmann, F., *Fedn Proc., Abstr.*, **32**, 489 (1973).
- ⁸ Redding, T. W., and Schally, A. V., *Proc. Soc. exp. Biol. Med.*, **131**, 420–425 (1969).
- ⁹ Tata, J. R., *Biochem. J.*, **104**, 1–10 (1967).
- ¹⁰ Guillemin, R., Yamazaki, E., Gard, D. A., Jutisz, M., and Sakis, E., *Endocrinology*, **73**, 564 (1963).
- ¹¹ Vale, W., Burgus, R., and Guillemin, R., *Proc. Soc. exp. Biol. Med.*, **125**, 210 (1967).
- ¹² Schally, A. V., and Redding, T. W., *Proc. Soc. exp. Biol. Med.*, **126**, 320 (1967).
- ¹³ Rapp, J. R., and Pyun, L. L., *Proc. Soc. exp. Biol. Med.*, **146**, 1021–1023 (1974).
- ¹⁴ Gershengorn, M. C., and Weintraub, B. D., *J. clin. Invest.*, **56**, 633–642 (1975).

Primer-dependent copying of rabbit globin mRNA with Q_{β} replicase

THE synthesis of complementary strands of heterologous RNAs has been achieved by incubating these RNAs with Q_{β} replicase in the presence of Mn^{2+} (refs 1, 2). Q_{β} replicase has been used in this way for the *in vitro* copying of RNAs for which no replicating enzymes are available. A further extension of such synthesis is offered by the recently discovered primer-dependent RNA synthesis by Q_{β} replicase³. This approach is specially suited for the copying of eukaryotic RNAs (mRNAs) with a 3'-terminating poly(A) sequence. The complementary RNA synthesis is started at these poly(A) sequences with oligo(rU)₆ as primer and continues into the heterologous part of the mRNAs. The particular advantages of this method are the independence of special structural features of the template RNA for the initiation of synthesis and the possibility to inhibit completely any disturbing 6S RNA synthesis which normally takes place in Q_{β} replicase incubations. Because of its defined start of synthesis, the newly synthesised complementary RNA may be very useful for sequence determinations of the template RNAs. I describe here experiments using rabbit globin mRNA as an example of the primer-dependent copying of eukaryotic mRNAs.

The standard conditions for these replicase incubations were as follows: Q_{β} replicase (preparation described in ref. 6) 80 U ml⁻¹, 12 mM MgCl₂, 80 mM Tris-HCl (pH 7.6), 0.8 mM ribonucleoside triphosphates, and rabbit globin mRNA (Searle) as well as oligo(rU)₆ (Boehringer) in concentrations as specified. Since endogenous 6S RNA synthesis is a major problem in Q_{β} replicase incubations with heterologous templates in the presence of the four common ribonucleoside triphosphates (ATP, UTP, CTP, GTP) (refs 4 and 5), conditions were sought which would inhibit this synthesis. 6S RNA synthesis is strictly dependent on the presence of GTP and it can be avoided completely if GTP is replaced by ITP in the incubation mixture (data not shown). This inhibition of 6S RNA seems to involve the initiation step, since the substitution of GTP by ITP does not interfere with the elongation steps of Q_{β} replicase reactions³. As the primer-dependent start of RNA synthesis is independent of GTP, GTP was replaced by ITP in the following enzyme assays, thus inhibiting the concomitant 6S RNA synthesis.

The kinetic analysis depicted in Fig. 1 shows that in such enzyme incubations the incorporation of ³H-CMP and ³H-AMP in response to globin mRNA as template is greatly stimulated

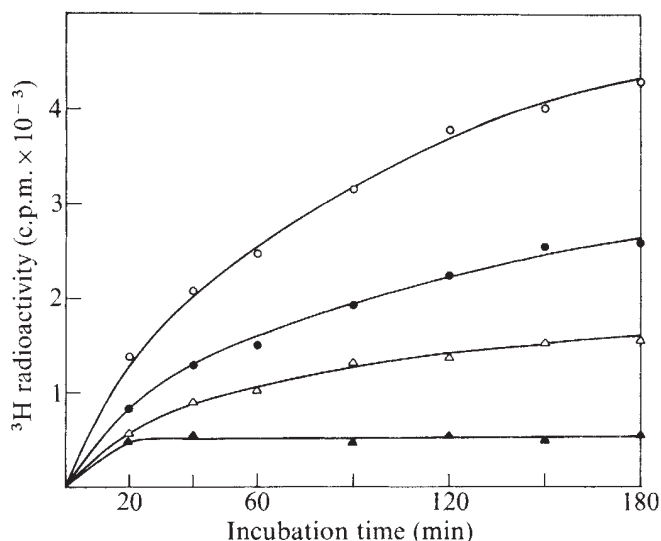


Fig. 1 Kinetics of oligo(rU)₆-dependent copying of rabbit globin RNA. Standard incubation mixtures (60 µl) containing ³H-CTP (7 × 10⁴ c.p.m. nmol⁻¹) and ³H-ATP (7 × 10⁴ c.p.m. nmol⁻¹), 40 µg ml⁻¹ rabbit globin RNA and no oligo(rU)₆ (▲), 70 µg ml⁻¹ oligo(rU)₆ (△), 140 µg ml⁻¹ oligo(rU)₆ (●), or 250 µg ml⁻¹ oligo(rU)₆ (○) were incubated at 30 °C. At the times indicated samples were taken (4 µl) and analysed for 6% trichloroacetic acid-insoluble radioactivity as described previously⁶.

by high concentrations of oligo(rU)₆. Incubations with ³H-CTP as the only radioactive substrate give the same result. It should be stressed that in addition to the great molar excess of primer, the template-enzyme ratio is also important for a successful synthesis. Incubations without template resulted in less than 5% of the RNA synthesis obtained in assays with template.

To ascertain the amount of globin-specific RNA in the total reaction product, renaturation tests were carried out after denaturation of the product. Table 1 shows that in the case of the product from a 3-h incubation, 49% of the incorporated radioactivity can be renatured to a double-stranded structure as analysed by RNase digestion in standard conditions. Some experiments yielded up to 80% of this double-stranded RNA. Addition of an excess of globin mRNA to the renaturation

Table 1 Renaturation of product RNA with template RNA

Sample	Time of annealing (min)	Resistance to RNase (%)
1 Non-denatured	0	51
2 Denatured	0	4
3 Denatured	5	28
4 Denatured + 25 µg ml ⁻¹ globin mRNA	5	42
5 Denatured + 125 µg ml ⁻¹ globin mRNA	5	45
6 Denatured + 125 µg ml ⁻¹ Q_{β} RNA	5	22
7 Denatured	30	36
8 Denatured + 125 µg ml ⁻¹ globin mRNA	30	49

A 100-µl standard incubation mixture containing ³H-CTP (1 × 10⁵ c.p.m. nmol⁻¹) and ³H-ATP (1 × 10⁵ c.p.m. nmol⁻¹), 40 µg ml⁻¹ globin RNA and 250 µg ml⁻¹ oligo(rU)₆ was incubated for 3 h at 32 °C, then brought to 1% SDS and 20 mM EDTA, phenolised, dialysed against 0.1 mM EDTA and concentrated to a small volume by desiccator evaporation. Aliquots containing 3,000 c.p.m. ³H-product were supplemented with additional RNAs as shown and heated for 1.5 min at 100 °C. The samples were then adjusted to 0.75 M NaCl and 0.075 Na-citrate in a total volume of 20 µl and annealed for the times indicated at 65 °C. After diluting the samples to 2 ml with 1 × SSC, the solutions were digested by RNase in standard conditions⁷ and analysed for acid-precipitable radioactivity⁸.

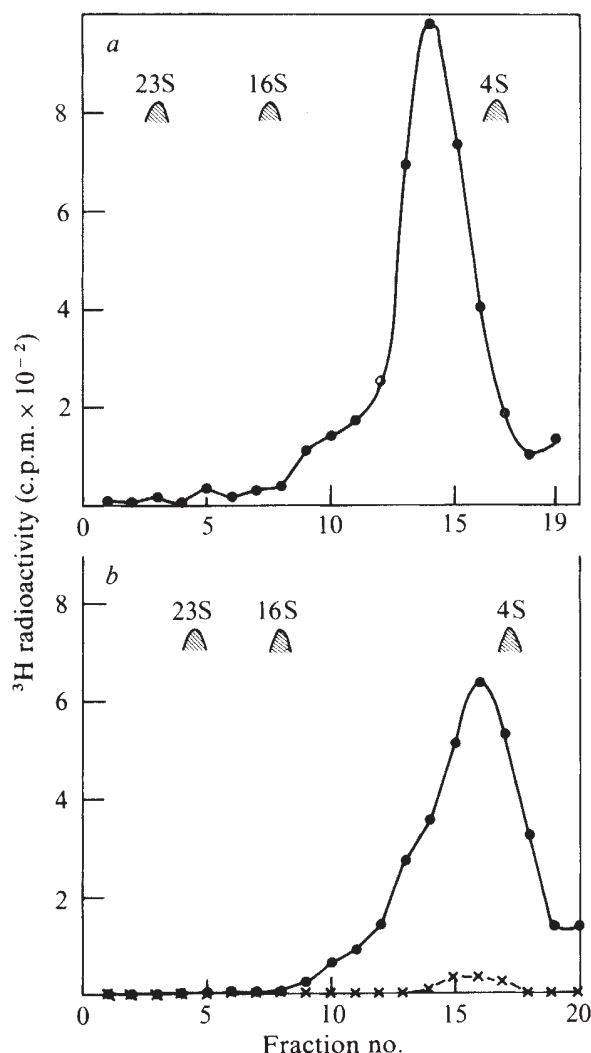


Fig. 2 Sedimentation analysis of the enzymatic product before and after denaturation. The SDS-treated and phenolised product of an incubation as described in Table 1 was adjusted to 0.5 M NaCl, heated for 5 min at 65 °C, diluted with and dialysed against the appropriate buffer for subsequent chromatography on a small cellulose column according to Franklin⁸, omitting the 30% ethanol step. The RNA-containing fractions eluted with TSE buffer⁴ (analysed by trichloroacetic acid precipitation of aliquots) were pooled, dialysed against 0.1 mM EDTA and concentrated to a small volume by desiccator evaporation. ³²P-labelled *E. coli* RNA was added and 20 µl of this solution layered directly (a) on a 5–23% linear sucrose gradient (0.1 M NaCl, 0.005 M EDTA, 0.05 M Tris-HCl pH 7.6). In b 30 µl of solution was diluted to 60 µl with H₂O, heated first for 1 min at 100 °C and quickly cooled before layering on a gradient. After centrifugation for 4 h at 64,000 r.p.m. in the SW 65 rotor of the Beckman centrifuge, the ³H- and ³²P-radioactivities of the fractions were counted after acid precipitation. One part of the fractions of b was treated with RNase in standard conditions before the acid precipitation and counting (×); the other part was analysed directly (●).

mixture speeded up the renaturation, but the presence of Q_B RNA had no effect on the reaction. These results suggest that part of the synthesised product is complementary to globin mRNA.

The unspecific RNA, synthesis of which increases with incubation time, is not identical with 6S RNA as judged from its sensitivity to RNase and from its S value of less than 4. The nature of this RNA was not investigated further, but it can easily be separated from the globin-specific RNA by chromatography on a cellulose column⁸. If an SDS-treated and phenolised reaction product is applied to such a column in the presence of 15% ethanol, the unspecific RNA does not absorb,

and the globin-specific RNA can be eluted only with buffer containing no ethanol. This latter RNA is RNase resistant and completely complementary to globin mRNA. It corresponds in quantity to the synthesised globin-specific RNA which was analysed directly in the enzymatic reaction product by renaturation. Therefore, cellulose column chromatography of the products of all primer-dependent reactions of Q_B replicase with heterologous RNAs as template, is recommended before further analysis.

The double-stranded material eluted from the cellulose column had an S value of about 7.5, as analysed by a sucrose gradient centrifugation in the presence of ³²P-labelled *E. coli* RNA as internal marker (Fig. 2a). After denaturation the S value of most of the material shifted to 5.6 with some RNA sedimenting as a shoulder at 10S (Fig. 2b). The relatively small size of most of the single-stranded RNA product seems to result from premature termination of synthesis on the template RNA, thus leading to a double-stranded structure with a single-stranded tail. This argument is favoured by the comparatively large S value of the double-stranded structure which gets smaller on RNase treatment specific for single-stranded RNA (data not shown). The reason for the unfinished synthesis in the case of rabbit globin RNA as template is unknown but it should be noted that in the Mn²⁺-type reaction of Q_B replicase with brome mosaic virus RNA as template, a smaller product of distinct size appeared in addition to the larger product¹.

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G. FEIX

*Institut für Biologie III,
University of Freiburg,
78 Freiburg i. Br.,
West Germany*

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- 1 Palmberg, A., and Kaesberg, P., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1371–1375 (1974).
- 2 Obinata, M., Nasser, D. S., and McCarthy, B. J., *Biochem. biophys. Res. Commun.*, **64**, 640–647 (1975).
- 3 Feix, G., and Hake, H., *Biochem. biophys. Res. Commun.*, **65**, 503–509 (1975).
- 4 Weissmann, C., Billetter, M. A., Goodman, H. M., Hindley, J., and Weber, H., *A. Rev. Biochem.*, **42**, 303–328 (1973).
- 5 Sumpter, M., and Luce, R., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 162–166 (1975).
- 6 Feix, G., and Sano, H., *Eur. J. Biochem.*, **58**, 59–64 (1975).
- 7 Billetter, M. A., and Weissmann, C., in *Procedures in Nucleic Acid Research* (edit. by Cantoni, G. L., and Davies, D.), 498 (Harper and Row, New York, 1966).
- 8 Franklin, R. M., *Proc. natn. Acad. Sci. U.S.A.*, **55**, 1504–1511 (1966).

Naturally occurring antibody against unusually high serum DNA polymerase activity

We have reported unusually high DNA polymerase activity in sera obtained from chimpanzees infected with the hepatitis B virus¹, and Kaplan *et al.*² reported hepatitis B-specific DNA polymerase activity in sera of human patients during post-transfusion hepatitis. We and others^{3–5} also found DNA polymerase activities associated with Dane particles, the putative hepatitis B virus, or their naked core components. Reports^{6,7} of the specific association of an e antigen-e antibody system with carriage of HBsAg prompted us to examine HBsAg positive sera containing unusually high levels of DNA polymerase activity for the presence of e antigen or e antibody. Subsequently, we studied the effects of sera containing e antibody on high DNA polymerase activity in sera from individuals with acute hepatitis B, chronic asymptomatic hepatitis B, chronic aggressive hepatitis B or chronic non-B hepatitis.

We have screened several hundred sera for high DNA polymerase activity using a standard radioisotope method⁸. Sera from normal healthy controls were found to contain low levels of polymerase activity, usually about 400 c.p.m.

Table 1 Effects of antisera on high serum DNA polymerase activity

Test serum No.	Patient serum No.	Serum description	Control activity (c.p.m.)	Adsorptions: c.p.m. % effect against control activity		
				anti-e	Adsorbed anti-e	anti-HB _s
1	1	HB _s Ag neg.	11,391†	6,706* /41% ↓	NT	NT
2	2	HB _s Ag neg.	33,311†	17,689‡ /47% ↓	35,076§ /5% ↑	44,979¶ /35% ↑
3	3	HB _s Ag neg.	19,564†	9,410* /52% ↓	NT	NT
4	4	HB _s Ag neg.	18,713†	NT	NT	16,000 /14% ↓
5	5	HB _s Ag neg.	95,432†	60,639‡ /36% ↓	99,978§ /5% ↑	91,644¶ /4% ↓
6	1:10 5§§	HB _s Ag neg.	11,412†	6,053‡ /47% ↓	9,672§ /15% ↓	11,223¶ /—
7	6	HB _s Ag neg.	4,229†	1,243* /71% ↓	NT	NT
8	7	HB _s Ag neg.	10,453†	3,856‡ /73% ↓	NT	NT
9	8	HB _s Ag pos. e neg.	881†	520* /36% ↓	NT	NT
10	9	HB _s Ag pos. e neg.	100,981†	88,625* /14% ↓	NT	104,270¶ /4% ↑
11	9	HB _s Ag pos. e neg.	100,981†	79,632‡ /22% ↓	NT	NT
12	10	HB _s Ag pos. e neg.	125,135†	NT	121,437** /3% ↓	NT
13	11	HB _s Ag pos. e Ag pos.	11,995†	NT	7,182,§ /40% ↓	NT
14	11	HB _s Ag pos. e Ag pos.	9,304†	3,157* /70% ↓	NT	NT
15	11	HB _s Ag pos. e Ag pos.	9,304†	5,358†† /51% ↓	NT	NT
16	12	HB _s Ag pos. e Ag pos.	7,128†	3,493* /50% ↓	NT	6,731¶ /6% ↓
17	13	HB _s Ag pos. e Ag pos.	4,125†	2,352‡ /43% ↓	NT	NT
18	14	HB _s Ag pos. anti-e pos.	57,847†	24,205‡ /58% ↓	NT	NT
19	15	HB _s Ag pos. anti-e pos.	34,614†	NT	NT	42,167 /22% ↑
20	15	HB _s Ag pos. anti-e pos.	33,012†	11,634* /65% ↓	NT	NT
21	15	HB _s Ag pos. anti-e pos.	26,257†	4,144‡ /84% ↓	NT	19,706¶ /25% ↓
22	16	HB _s Ag pos. anti-e pos.	17,103‡‡	6,144‡ /66% ↓	NT	18,473¶ /8% ↑
23	16	HB _s Ag pos. anti-e pos.	17,103†	6,430†† /64% ↓	NT	NT
24	17	HB _s Ag pos. anti-e pos.	8,724†	NT	5,928** /32% ↓	NT
25	1:3 17¶¶	HB _s Ag pos. anti-e pos.	5,350‡‡	NT	4,171§ /22% ↓	NT
26	18	HB _s Ag pos. anti-e pos.	874†	693‡ /21% ↓	NT	NT
27	19	HB _s Ag pos. anti-e pos.	36,872†	20,044* /46% ↓	NT	37,745 /2% ↑

DNA polymerase activity in serum was determined by a radioisotope method that measures the incorporation of ³H-dTTP into an acid-insoluble product. The polymerase assay contained in a volume of 0.370 ml: 50 µl of serum and 260 µl 0.01 M Tris buffer, pH 7.8, containing 30 mM MgCl₂·6H₂O, 60 mM KCl, and 1.5% Triton X-100; the balance of the reaction mixture contained (final assay concentrations) mercapto-ethanol (2.5 mM), dATP, dCTP, dGTP (0.25 mM each), 50 µg carrier chicken cell DNA and ³H-dTTP (0.90 µM, specific activity 54 Ci mmol⁻¹). All reaction mixtures were incubated for 3 h at 37 °C; two 100-µl samples per assay were pipetted on to Whatman 3MM filter paper disks, which were then transferred to a solution of ice-cold 5% trichloroacetic acid (TCA) plus 2% pyrophosphate. The disks were washed four times with this solution, once with a 5% TCA solution, twice with 95% ethanol and then air dried. Each disk was counted in 10 ml of a toluene PPO (PPO = 2,5-diphenyloxazole)-POPOP (POPOP = 1,4-di-[2-(5-phenyloxazolyl)]-benzene) cocktail. Zero-time (*T* = 0 min) c.p.m. were typically 100 and were subtracted from the gross sample c.p.m. to yield the tabulated c.p.m. HB_sAg and anti-HB_s content of sera were determined by solid phase radioimmunoassay techniques, using commercial reagents (Abbott). Measurement of e antigen and antibody was performed using rheophoresis plates (Abbott) with incubation at 27 °C in humidified Petri dishes. The standard e antigen and e antibody reagents used were obtained from HB_sAg positive blood donors. All adsorptions were carried out at 37 °C for 1 h, followed by overnight incubation at 5 °C (16 h). Test sera (50 µl each) were adsorbed with 50 µl normal human serum and centrifuged the following day at 5,000g for 30 min to pellet out immune aggregates; the supernatants were used as the control sera in these experiments. Adsorptions of test sera with anti-e and anti-HB_s antisera were carried out in a similar manner. Anti-e antiserum (100 µl) was preadsorbed with 10 µl of either goat anti-human immunoglobulin A, G and M antiserum (or with horse antihuman IgG antiserum) before use as an adsorbant of the test serum. Conditions for preadsorption of the anti-e antiserum were as described above, except that overnight incubation was omitted. Preadsorption of normal human serum with either goat or horse antiserum before use as an adsorbant of test sera did not alter control sera DNA polymerase activity. NT, not tested.

*Anti-e antibody positive serum No. 1 (human).

†Normal human serum No. 1 (NHS).

‡Anti-e antibody positive serum No. 2 (human).

§Goat anti-human immunoglobulin A, G and M antiserum.

¶ Anti-HB_s antiserum (human).

|| Anti-HB_s antiserum (chimpanzee).

**Horse anti-human IgG antiserum (H and L chain specific).

††Anti-e antibody positive serum No. 3 (human).

‡‡Normal human serum No. 2.

§§1:10 dilution of test serum No. 5 in normal human serum.

¶¶1:3 dilution of test serum No. 17 in normal human serum.

|||Patient sera Nos 14, 15, 16, 17 and 18 represent serial bleedings of a single patient.

above background, whereas sera from acute or chronic cases of hepatitis B ranged in activity from 450 to 1,500 c.p.m. Several sera, however, had unusually high levels of DNA polymerase, ranging in activity from 4,000 to 173,000 c.p.m. All the sera used in our study were selected initially on the basis of very high DNA polymerase activity, without previous regard to the presence of e antigen or e antibody (Table 1). With these sera we found no correlation between this high DNA polymerase activity and the presence or absence of HB_sAg, e antigen or e antibody. It is interesting that high DNA polymerase activity was found in sera from individuals with chronic non-B hepatitis as well as in two individuals who had e antibody.

The inhibitory effect of serum containing e antibody on the DNA polymerase activity found in the above sera was unexpected. This inhibitory effect was not confined to serum samples from hepatitis B patients, since high DNA polymerase activity in sera from chronic non-B hepatitis patients was similarly suppressed by addition of e antiserum (for example, serum containing e antibody). Briefly, sera containing high DNA polymerase activity were mixed with either normal human serum (the control) or human serum containing e antibody or anti-HB_s and incubated for 1 h at 37 °C and overnight at 5 °C; the mixtures were then centrifuged at 5,000g for 30 min. The supernatants were assayed for residual DNA polymerase activity. Substantial reductions in polymerase activity (up to 84%) were found in serum samples incubated with e antiserum in contrast to samples reacted with normal human serum or anti-HB_s. For example, when two serum samples with control DNA polymerase activities of 9,300 and 26,500 c.p.m. were adsorbed with e antiserum, their polymerase activities were reduced to 3,150 and 4,140 c.p.m. respectively. This phenomenon was confirmed by repeating the assays using two other sera containing e antibody for the pre-incubation step. Sera containing anti-HB_s antibody did not affect substantially any of the high DNA polymerase activities, indicating that the activity was not associated with HB_sAg. These findings strongly suggested the presence of a specific factor in serum containing e antibody that can inhibit DNA polymerase activity. Adsorption of e antiserum with goat anti-human IgG antiserum inactivated this factor, indicating that it was an antibody.

Statistical analysis of the effects of e antiserum, adsorbed e antiserum, and anti-HB_s antiserum on test serum DNA polymerase activity was performed by first ranking the percentage effects (calculated as percentage depression or increase over control activity) of the antisera on the test serum activity and then inspecting the resulting array for possible separation points. Such a point occurred at 35% depression. Seventeen of the twenty-one test sera contained DNA polymerase activity which could be depressed by 36–84%. Only one of seven test sera examined contained DNA polymerase activity which could be depressed more than 36% (40%) by the e antiserum which had been pre-adsorbed with goat anti-human IgG. None of the ten anti-HB_s adsorbed test sera had depressions (or increases) in polymerase activity $\geq 36\%$ of the matching control activity. Student's *t* test was applied to the difference between proportions exceeding 35% depression (or increase) in activity to determine the statistical significance of the findings. For the e antiserum proportion (17/21) compared with adsorbed e antiserum proportion (1/7), the probability of e antiserum producing an excess of depressions on the basis of chance alone is $P \leq 0.001$ ($t_{36}=3.85$), strongly suggesting the involvement of an antibody (IgG) in the suppression of DNA polymerase activity. Similarly, the probability of producing an excess of depressions compared with anti-HB_s (0/10) is $P < 0.001$ ($t_{29}=6.31$), indicating the absence (in the two different anti-HB_s antisera used) of a naturally occurring antibody against DNA polymerase activity in the test sera. The significance of these findings was basically unchanged

when the effects of the antisera on high polymerase activity were analysed statistically using results from individual patient's sera instead of test sera. In this case, 14 of 16 patients' sera contained DNA polymerase activity which could be depressed 36–84% by adsorption with e antiserum.

As far as we know, this is the first report of a naturally occurring antibody which specifically inhibits high DNA polymerase activity in human serum. In addition, the inhibitory effect of e antiserum was not confined to DNA polymerase activity associated with HB_sAg positive sera, suggesting a more general role of the inhibitory factor (now believed to be an antibody) in suppressing unusually high DNA polymerase activity. These data do not show whether or not the inhibitory effect of e antiserum directly involves the DNA polymerase enzyme or its template. Furthermore, the polymerase activities reported here have not been characterised as specific to either host or virus; however, it is tempting to speculate on the existence of naturally acquired (occurring) anti-viral DNA polymerase antibodies which can either abort or modify the course of a viral infection or virus-induced disease.

D. W. BRADLEY
B. L. MURPHY
J. L. SMITH
J. E. MAYNARD

Phoenix Laboratories Division,
Center for Disease Control,
4402 North Seventh Street,
Phoenix, Arizona 85014

G. L. GITNICK

Department of Medicine,
School of Medicine,
University of California Los Angeles Center
for the Health Sciences,
Los Angeles, California 90024

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- ¹ Bradley, D. W., Maynard, J. E., Berquist, K. R., and Krushak, D. H., *Nature*, **251**, 356–357 (1974).
- ² Kaplan, P. M., Gerin, J. L., and Alter, H. J., *Nature*, **249**, 762–764 (1974).
- ³ Kaplan, P. M., Greenman, R. L., Gerin, J. L., Purcell, R. H., and Robinson, W. S., *J. Virol.*, **12**, 995–1005 (1973).
- ⁴ Robinson, W. S., and Greenman, R. L., *J. Virol.*, **13**, 1231–1236 (1974).
- ⁵ Hirschman, S. Z., Gerber, M., and Garfinkel, E., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3345–3349 (1974).
- ⁶ Magnus, L. O., and Espmark, J. A., *J. Immun.*, **109**, 1017–1021 (1972).
- ⁷ Nielsen, J. O., Dietrichson, O., and Juhl, E., *Lancet*, **ii**, 913–915 (1974).
- ⁸ Robinson, W. S., and Robinson, H. L., *Virology*, **44**, 457–462 (1971).

Safer derivatives of bacteriophage λ gt $\cdot$ λ C for use in cloning of recombinant DNA molecules

THOMAS *et al.*¹ have constructed a mutant strain of bacteriophage λ (λ gt $\cdot$ λ C) which is especially well suited to studies involving the cloning of recombinant DNA molecules. Specifically, this bacteriophage, deleted of 15% of its genome, has only two *EcoRI* restriction endonuclease sites which, when cleaved, reduce the phage chromosome to three DNA fragments. The larger of these, the right- and left-hand arms of the chromosome, encode all functions necessary for lytic infection. The smaller, central fragment consisting of approximately 5,000 base pairs encodes the λ attachment site, the *Int* and *Xis* functions and a necessary part of the λ -generalised recombination system, but no genes necessary for phage propagation. Although left- and right-hand restriction fragments have all necessary λ genes, they seem to lack sufficient length of DNA for packaging into a viable phage particle¹. This provides a powerful positive selection for recombinant DNA molecules consisting of isolated and rejoined right and left restriction fragments

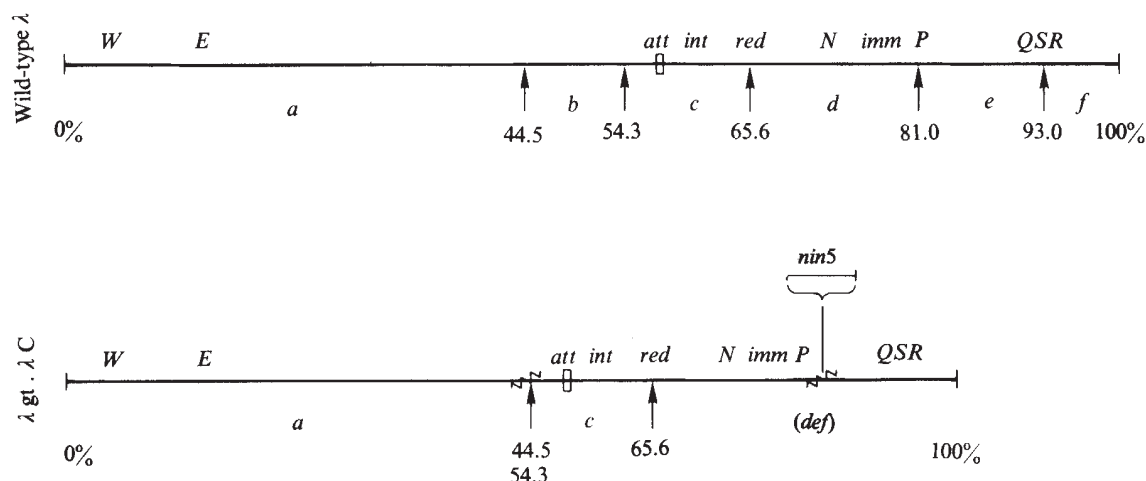


Fig. 1 Characteristics of wild-type λ and λ gt- λ C. *Eco*RI target sites given below line as % λ unit length¹². *Eco*RI fragments are lettered *a* to *f* below line, λ genes above line. For λ gt- λ C note that *b* fragment is removed and a hybrid RI target at 54.3% is regenerated¹. Amber mutations in gene *S* were isolated by modifying the procedure of Goldberg and Howe⁷ as follows. λ gt- λ C lysogens of *E. coli* *mutD*³ were obtained by isolating colonies from the centre of a phage plaque. λ -resistant clones of these lysogens were made by plating on EMBO plates seeded with λ vir (ref. 8); 10-ml cultures of these λ -resistant, λ gt- λ C lysogens were grown at 32 °C in tryptone broth to about 2×10^8 cells ml⁻¹. Cultures were induced by growing at 42 °C for 20 min and then at 37 °C for 3 h. Lysis was virtually complete by 60 min. After 3 h at 37 °C, culture was centrifuged for 10 min at 4,000 r.p.m. in a Sorval GLC-2. Pellet containing unlysed cells and debris was washed once with tryptone broth and centrifuged again. Pellet was resuspended in 0.5 ml tryptone broth containing enough λ antiserum to give a *K* value of 1. This mixture was incubated at 32 °C for 5 min and then diluted 100-fold in 0.01 M Tris, 0.01 M MgSO₄, 0.1% gelatin (TMG). After centrifugation at 4,000 r.p.m. for 15 min, the pellet was washed with 45 ml TMG and re-centrifuged. This pellet was resuspended in 1 ml TMG and incubated with several drops of chloroform at 37 °C for 5 min. The chloroform-treated mixture was plated on a mixed indicator of *supO* (N99) and *supF* (*Ymel*) bacteria at 37 °C. Turbid plaques, indicating growth on one indicator but not the other, appeared at a frequency of 10^{-3} – 10^{-4} . Two isolates were characterised by growth on suppressor strains and by complementation as described by Shimada *et al.*⁸. Both mutants formed plaques on *supF* bacteria but plated at less than 10^{-6} efficiency on *supO* hosts. In addition, both mutants plated well on *supC* (CA169) but grew poorly on *supE* (C600). Both isolates complemented all tested λ mutants except *Sam7*. The mutants were therefore designated *Sam100* and *Sam101*. λ Sam100 lysogens of *supO* hosts do not lyse after induction until chloroform is added. λ Sam100 lysogens of *supE* hosts behave similarly but 2 h after induction, some spontaneous lysis can be observed. Optimal phage yields (about 200–500 phage per induced cell) from induced *supE* lysogens are obtained by collecting phage 2 h after induction.

which have also incorporated a fragment of foreign DNA restoring the λ chromosome to viable size.

Although the λ gt- λ C-*Escherichia coli* K12 host-vector system offers rather special advantages as a system in which to clone DNA fragments that might contain potentially biohazardous segments, the need for further modification to make this and other vectors even safer was made clear during the Asilomar Conference on Recombinant DNA Molecules². Accordingly, we have introduced three muta-

tions into the original λ gt- λ C phage which reduce the possibility of its encountering a host in nature by at least a factor of 10^6 and which make it especially amenable to high titre growth in small and therefore more manageable volumes of medium. These modifications should make this phage suitable for use in experiments which require moderate biological containment barriers².

Here we describe the construction of derivatives of λ gt- λ C containing three amber mutations, *Sam100* *Eam1100* and

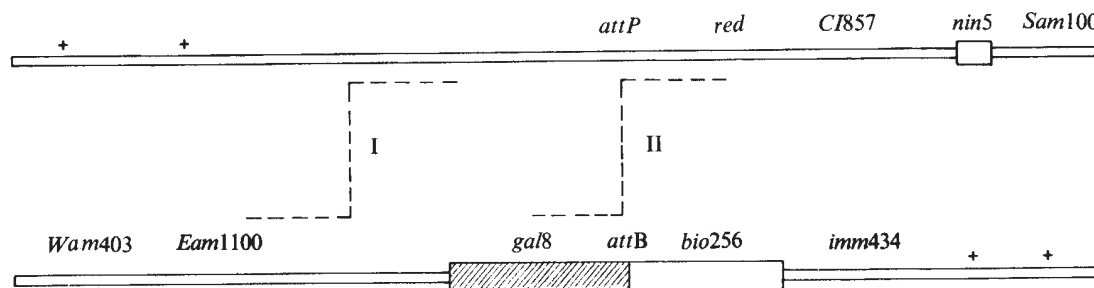


Fig. 2 Construction of λ gt- λ C *Wam403 Eam1100 Sam100*. Phage parent I (a) was λ gt- λ C *Sam100* obtained as described previously. Phage parent II (b) (obtained as G96 from NIH stock collection) was λ *Wam403 Eam1100 gal8 bio256 imm434 clt1*. Strain *Ymel* was infected with both parents at a multiplicity of five of each phage. After 15 min adsorption at 32 °C, the infected cells were treated with ultraviolet light at a dose of 300 erg mm⁻², diluted 1,000-fold in tryptone broth containing 10^{-2} M MgSO₄, and incubated at 40 °C for 70 min. Chloroform was added to complete lysis. Incubation at 40 °C insured a low level of *Int*-promoted recombination⁹. Dilutions of the lysate were plated on *Ymel* *recA56*. Parent I, but not parent II, will make plaques on this host because *bio256* deletes the *red* and *gam* genes¹⁰. These plaques were tested on *Ymel* *groE*¹¹. Only parent II and recombinants carrying the *Eam1100* mutation will plate on this host. The presence of the *Wam403* mutation does not influence this test. Several isolates that made plaques on *Ymel* *recA56* and on *Ymel* *groE* were tested for their immunity by ability to grow on λ and phage 434 lysogens of *Ymel*. Because both *Eam1100* and *Wam403* are suppressed well on *supE* hosts but *Sam100* is not, the presence of *Sam100* was inferred by poor growth on C600. The presence of the *gal8* substitution was determined by measuring transduction to *gal*⁺ of a *Ymel* *gal* deletion (SA825). Stocks with λ immunity also made clear plaques above 37 °C and turbid plaques at 32 °C indicating the presence of the *CI857* mutation. Complementation tests⁸ were carried out to confirm that the phage contained *E*, *W* and *S* mutations. Finally, the presence of *nin5* was determined by plating on *E. coli* 'super nus' (M. Baumann and D. I. Friedman; N. S., unpublished). Two isolates were chosen for further study. One transduced SA825 to *gal*⁺ and the other did not. Both were immunity λ , *E*⁺, *W*⁺ and *S*⁺. The *gal*⁺ recombinant presumably resulted from an event depicted by crossover I, the *gal*⁺ recombinant from crossover II.

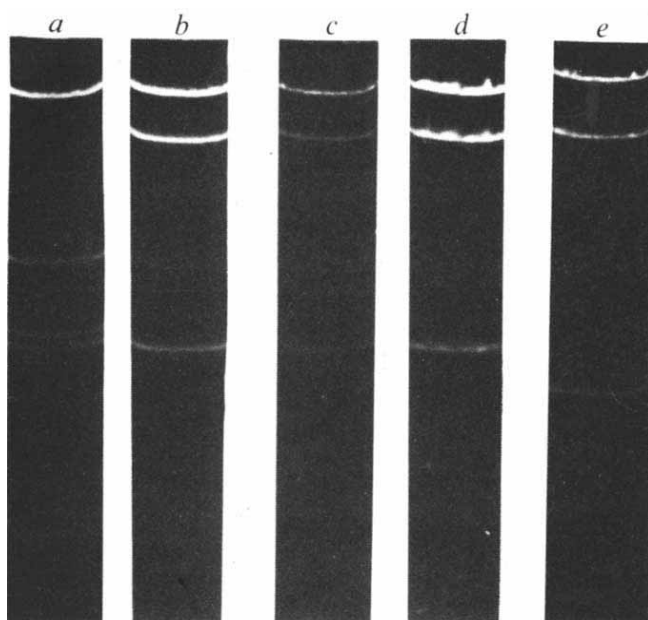


Fig. 3 Electrophoretic separation of *EcoRI* fragments of DNA from wild-type λ and the λ gt- λ C derivatives. *a*, λ c1857*Sam7*; *b*, λ gt- λ C; *c*, λ gt- λ C *Sam100*; *d*, λ gt- λ C *Wam403 Eam1100 Sam100*; *e*, λ gt- λ C *Wam403 Eam1100 Sam100 gal8*. Phage were prepared from induced lysogens or plate stocks⁵. Phage suspensions of $1-20 \times 10^{10}$ phage ml⁻¹ were adjusted to a density of 1.35 g cm^{-3} by addition of 0.5 g CsCl per 1.0 ml suspension and concentrated by centrifugation on a layer of CsCl at 1.70 g cm^{-3} in 20 mM Tris-HCl, pH 7.8, and 10 mM MgCl₂ in an SW41 rotor for 1.0 h at 30,000 r.p.m. at 4 °C. The interface bands were pooled and banded to equilibrium by centrifugation in an SW56 rotor for 18 h at 35,000 r.p.m. at 4 °C. The suspensions were stored in CsCl at 0–5 °C. Phage suspensions ($1-50 \times 10^{11}$ phage ml⁻¹) in CsCl were brought to 1.0 mg ml^{-1} Pronase (Calbiochem, nuclease-free) and dialysed at 37 °C for 90 min against 100 volumes buffer A (20 mM Tris-HCl, pH 7.8; 1.0 mM EDTA, 0.1 M NaCl) plus 0.002% Triton X-100. After gentle extraction with an equal volume of buffer A-saturated phenol, DNA was dialysed for 2–3 h against 100 volumes buffer A plus 0.002% Triton X-100, and then overnight against 100 volumes buffer A. The resulting DNA ($0.010-1.2 \text{ mg ml}^{-1}$) is stored at 0–5 °C. *EcoRI* was purified from the strain *E. coli* BRY 13.3/1100.5 (described elsewhere). The standard *EcoRI* reaction contained 0.1 M Tris-HCl, pH 7.9, 50 mM NaCl, 0.1 mM EDTA, 12 mM MgCl₂, $10-50 \text{ } \mu\text{g ml}^{-1}$ DNA, and enough *EcoRI* to completely digest 30–150 $\mu\text{g } \lambda$ DNA. The reaction was incubated for 30 min at 37 °C and stopped by addition of EDTA to 25 mM. After incubation for 5 min at 75 °C, the reactions were electrophoresed through 0.7% Agarose slab gels as described previously⁶. The DNA was stained by immersion of the gel in 0.0025% ethidium bromide for 3 min and visualised under an ultraviolet lamp.

mutations separately and found them to be of the order of 10^{-6} . The *W* and *E* mutations therefore have an expected joint reversion frequency to *am*⁺ of approximately 10^{-12} . The probability of a host amber suppressor mutation arising spontaneously is approximately $10^{-7}-10^{-10}$ (ref. 4).

Two phage derivatives obtained from the cross shown in Fig. 2 have been characterised genetically by complementation tests and biochemically by *EcoRI* fragment patterns. The first, λ gt- λ C *Wam403 Eam1100 Sam100*, has the restriction pattern characteristic of the parent phage, λ gt- λ C (Fig. 3). The second, λ gt- λ C *Wam403 Eam1100 Sam100 gal8*, has a larger left-arm fragment following *EcoRI* restriction and a smaller central fragment. In a separate mixing experiment with λ c1857, this latter fragment's molecular weight was found to be 3.2×10^6 . We conclude that there is a single *RI* cleavage site within the *gal8* substitution.

The presence of the *gal8* substitution in the second phage enables establishment of lysogeny by recombination with the host genome and facilitates recognition of such lysogens. Although this is not desirable in recombination experiments with an uncharacterised constellation of DNA sequences, it may prove useful in the handling of a homogeneous, well-characterised fragment.

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L. ENQUIST
D. TIEMEIER
P. LEDER
R. WEISBERG
N. STERNBERG

Laboratory of Molecular Genetics,
National Institute of Child Health and
Human Development,
National Institutes of Health,
Bethesda, Maryland 20014

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- 1 Thomas, M., Cameron, J. R., and Davis, R. W., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 4579–4583 (1974).
- 2 Science, **188**, 991 (1975).
- 3 Fowler, R. G., Degnen, G. E., and Cox, E. C., *Molec. gen. Genet.*, **133**, 179–191 (1974).
- 4 Wilson, J. H., and Kells, S., *J. molec. Biol.*, **69**, 39–55 (1972). Comer, M. M., Guthrie, C., and McClain, W. H., *J. molec. Biol.*, **90**, 665–681 (1974).
- 5 Schrenk, W. J., and Weisberg, R. A., *Molec. gen. Genet.*, **137**, 101–107 (1975).
- 6 Helling, R. B., Goodman, H. M., and Boyer, H. W., *J. Virol.*, **14**, 1235–1244 (1974).
- 7 Goldberg, A. R., and Howe, M., *Virology*, **38**, 200–202 (1969).
- 8 Shimada, K., Weisberg, R. A., and Gottesman, M. E., *J. molec. Biol.*, **63**, 483–503 (1972).
- 9 Guarneros, G., and Echols, H., *Virology*, **52**, 30–38 (1973).
- 10 Zissler, J., Signer, E., and Schaefer, F., *The Bacteriophage Lambda* (edit. by Hershey, A. D.), 455–468 (Cold Spring Harbor Laboratory, New York, 1971).
- 11 Sternberg, N., *J. molec. Biol.*, **76**, 1–23 (1973).
- 12 Murray, N. E., and Murray, K., *Nature*, **251**, 476–481 (1974).

Construction of infectious polyoma hybrid genomes *in vitro*

WITH recent advances in nucleic acid biochemistry, it has become possible to recombine different animal viral DNAs at specific sites *in vitro*^{1,2}. Specific hybrid viral genomes can be used in the genetic analysis of animal tumour viruses. With this aim in mind, we have made hybrids of two strains of polyoma (A-3 and P16). A-3 and P16 were developed from independent isolates and have been characterised both phenotypically and genotypically^{3,4}. Using the hybrids it has been possible to begin to locate the DNA regions coding for phenotypic differences in the parent strains.

The two viruses differ phenotypically in their plaque morphology and haemagglutination (HA) properties; P16 produces small plaques and agglutinates guinea pig red blood cells at 20 °C as well as 4 °C whereas A-3 produces large plaques and agglutinates guinea pig red blood cells at 4 °C but not at 20 °C. The P16 and A-3 strains also differ genotypically. Three genotypic differences are readily dis-

Wam403. The *S* mutation, which prevents host lysis, was isolated by mutagenesis because the location of an *RI* cleavage site very close to or within the *S* gene makes it difficult to cross an *Sam* mutation into λ gt- λ C without introducing unwanted *RI* sites (Fig. 1). λ Sam100 is not efficiently suppressed in conditions that suppress both *E* and *W* amber mutations. This produces an additional advantage in that one can obtain large phage concentrations in small, easily manipulated volumes of media. *Eam1100* and *Wam403* were crossed into λ gt- λ C. The *E* gene product is the major head protein of the phage. The *W* gene product enables addition of tails to heads. Thus, λ carrying *Eam1100* cannot make phage heads, whereas λ carrying *Wam403* in the presence of a functional *E* gene, makes heads and tails but does not assemble these components. An additional effect of the *Eam1100* mutation is that oligomeric λ DNA, the product of intracellular phage replication, is not cut into monomeric DNA with normal cohesive ends. We have tested the reversion frequency of each of these amber

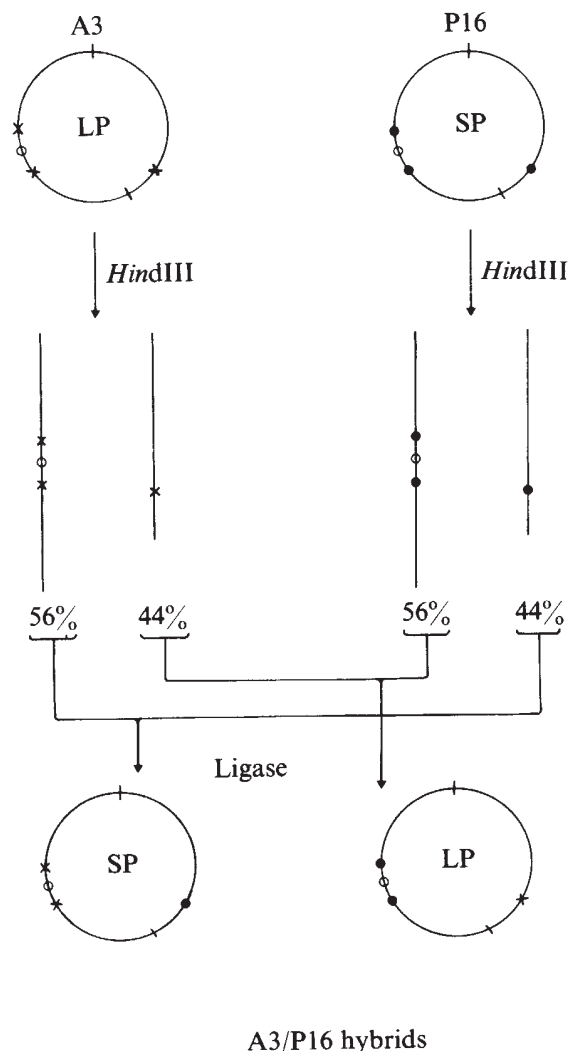


Fig. 1 Construction of DNA hybrids *in vitro*. The A-3 and P16 strains of polyoma virus vary genotypically in at least three different sites denoted by (x) for A-3 and (•) for P16 on the circular genomes where (o) marks the origin and (→) marks the *Hind*III restriction endonuclease cleavage sites. The two strains also differ phenotypically; A-3 has a large plaque (LP) morphology whereas P16 has a small plaque (SP) morphology. Purified supercoiled (FI) DNA of A-3 and P16 viruses were digested with *Hind*III to yield two fragments approximately 44% and 56% of the genomes. The *Hind*III fragments from each viral DNA were separated by electrophoresis through 1.4% Agarose gels¹¹, visualised by ethidium bromide staining^{11,12}, individually sliced from the gels, eluted by gel electrophoresis and concentrated by ethanol precipitation. The A-3 *Hind*III-56% fragment was combined in a test tube with the P16 *Hind*III-44% fragment while the P16 *Hind*III-56% fragment was combined in another test tube with the A-3 *Hind*III-44% fragment. The fragments were covalently joined with *T*₄ DNA ligase in a reaction mixture containing 67 mM Tris (pH 7.6), 6.7 mM MgCl₂, 0.67 mM dithiothreitol, 0.067 mM rATP, 300 µg per ml of each DNA fragment and 2×10^{-3} units *T*₄ DNA ligase by incubation at 5 °C for 16 h. Subsequent infection of mouse embryo cells with the hybrid DNAs for plaque assay facilitated the determination of the plaque morphology characteristics of the hybrid strains (LP and SP as indicated).

cerned in the *Haemophilus parainfluenzae* restriction endonuclease (*Hpa*II) cutting patterns of their DNA (Fig. 2a and ref. 3). The most obvious difference between the P16 and A-3 *Hpa*II cleavage patterns is that P16 is cleaved nine times by *Hpa*II whereas A-3 is cleaved only eight times. The additional cleavage site of P16 is located within the largest *Hpa*II fragment of A-3 (designated A-3 *Hpa*II-1) and generates two new fragments (designated as P16 *Hpa*II-1a and P16 *Hpa*II-1b) which migrate in 3.3% polyacrylamide-0.5% Agarose gels on either side of the A-3

*Hpa*II-fragment-4 (*Hpa*II-4). In addition, the *Hpa*II-3 and *Hpa*II-5 fragments of A-3 are slightly smaller (migrate faster) than the equivalent P16 fragments (designated P16 *Hpa*II-3 and P16 *Hpa*II-5). Hybrids constructed from these two polyoma strains can therefore be studied both at the genotypic level and at the phenotypic level; hybrid formation can be confirmed by the *Hpa*II cleavage patterns of the hybrid viral DNAs and the phenotypic differences of plaque size and HA ability can potentially be correlated with a specific region of the viral genome.

The construction of A-3 and P16 hybrid viruses is schematically shown in Fig. 1. Both A-3 and P16 are cleaved twice by the *Hind*III restriction endonuclease yielding two fragments of approximately 44% and 56% (designated *Hind*III-44% and *Hind*III-56% respectively) of the genome³. These fragments were purified by gel electrophoresis through 1.4% Agarose gels. The *Hind*III-44% of A-3 was combined with the *Hind*III-56% of P16 and, vice versa, the P16 *Hind*III-44% was combined with the A-3 *Hind*III-56%. To encourage intermolecular rather than

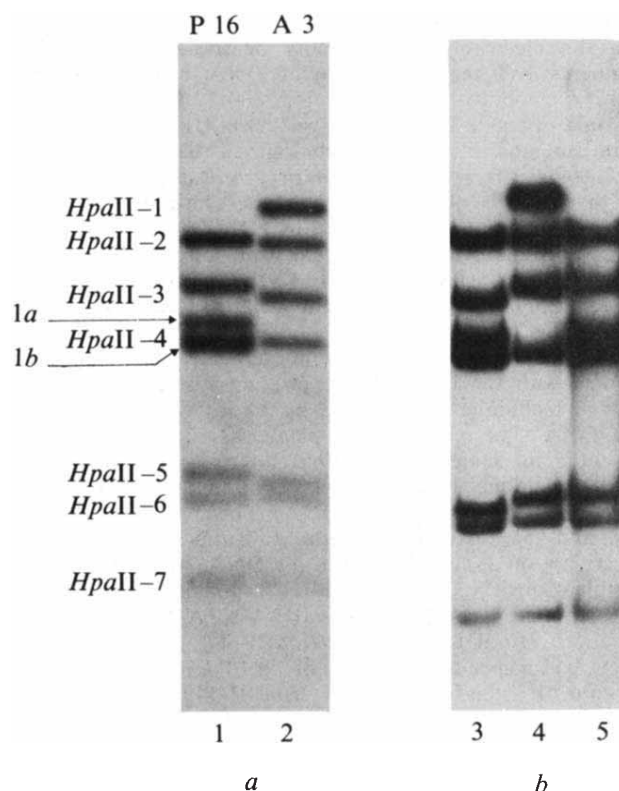


Fig. 2 a, An autoradiograph showing the restriction nuclease cleavage patterns of (1) ³²P-labelled P16 DNA and (2) ³²P-labelled A-3 DNA (modified from Fried *et al.*³). The numbers at the side of the gel designate the nomenclature of the bands. The gels (20 × 20 cm) were composed of 3.3% acrylamide-0.17% bis-acrylamide-0.5% Agarose and run as described previously⁷. In P16, *Hpa*II-1 is absent and two new fragments (1a and 1b) are present. The sizes of *Hpa*II-3 and *Hpa*II-5 can be seen to vary between the A-3 and P16 strains. b, Secondary whole mouse embryo cells (WME) were infected with each A-3/P16 hybrid DNA, overlaid with agar and stained with neutral red after 9 d incubation at 37 °C. Plaques of each hybrid were picked, virus stocks of each plaque were produced at low multiplicity¹⁰ and ³²P-labelled form I DNA was prepared as previously described^{3,7}. The labelled DNAs were digested with *Hpa*II and the resulting fragments were electrophoresed through a 3.3% acrylamide-0.17% bis-acrylamide-0.5% Agarose slab gel. The autoradiograph shows the representative *Hpa*II cutting pattern of (3) the small plaque hybrid P16 *Hind*III-44%+ A-3 *Hind*III-56%, (4) the large plaque hybrid A-3 *Hind*III-44%+ P16 *Hind*III-56%, and (5) small plaque P16 control. For both the hybrids, five plaques were picked and tested. Five out of five plaques in each case showed the expected cutting patterns. In the gels in a and b *Hpa*II-8 has been run off the gel. There was no difference in the electrophoretic mobility of *Hpa*II-8 between the A-3 and P16 strains or any of the plaque isolates.

Table 1 Analysis of the phenotypic properties of A-3/P16 hybrids

DNA	Plaque size*	HA at 20 °C†	HA at 4 °C
A-3 Form I DNA	LP	—	+
P16 Form I DNA	SP	+	+
A-3 <i>HindIII</i> -44%+P16 <i>HindIII</i> -56%	LP	—	+
P16 <i>HindIII</i> -44%+A-3 <i>HindIII</i> -56%	SP	+	+

*LP, large plaque morphology; SP, small plaque morphology⁴.

†Ability of virus stocks derived from picked plaques to haemagglutinate guinea pig red blood cells at 20 °C.

(—) indicates less than 10 HA were detectable; (+) indicates HA titres between 160 and 1280. There was no difference in the HA titres of the P16 type at 4 and 20 °C.

intramolecular joining of fragments, each DNA was present at a concentration of 300 µg ml⁻¹. The combined fragments were first heated to 50 °C to ensure complete denaturation of the four-base overlap at the restriction nuclease cutting site⁵ and then cooled slowly to 5 °C and allowed to re-anneal at 5 °C for 30 min. The resulting hybrid DNAs were covalently closed by T₄ DNA ligase (a gift from Dr Ian Molineux). In the conditions used, approximately 90% of the DNA molecules were judged to have been ligated. This was determined by observing the disappearance of molecules with the electrophoretic mobility of the 44% and 56% fragments and the appearance of DNA with an electrophoretic mobility for molecules 88–112% the size of polyoma. Low levels of unligated *HindIII*-44% and 56% fragments and intramolecularly ligated *HindIII*-44% and 56% fragments were observed in gels but accounted for less than 10% of the ligation products. The remaining 90% of the DNA products migrated in 1.4% Agarose gels with a mobility equivalent to linear and circular forms of molecules 88%, 100% and 112% the length of polyoma DNA. Random intermolecular associations account for the size distribution of products.

Secondary whole mouse embryo cells (WME) were infected with the hybrid DNA products using the DEAE-dextran technique as described by Thorne *et al.*⁶ for polyoma DNA. The results of the plaque assays with DNA are presented in Table 1. The *HindIII*-44% and *HindIII*-56% fragments alone had negligible contaminating infectivity (less than 1 × 10² PFU per µg DNA). The combined *HindIII*-44% and -56% fragments without ligation had a specific infectivity of 3 × 10³ PFU per µg DNA; thus the cell is capable of ligating the two *HindIII* fragments at a low level. The *in vitro* ligated P16 *HindIII*-44%/A-3 *HindIII*-56% and A-3 *HindIII*-44%/P16 *HindIII*-56% ligated hybrid DNAs had a specific infectivity of 1 × 10⁵ PFU per µg DNA. In control experiments, A-3 *HindIII*-44%/A-3 *HindIII*-56% ligated DNA also had a specific infectivity of 1 × 10⁵ PFU per µg DNA indicating that the hybrid viral DNAs are as efficient in plaque formation as *in vitro* constructed parental DNA. Untreated supercoiled FI polyoma DNA or singly-nicked FII DNA had a specific infectivity of 1 × 10⁶ PFU per µg DNA. Analysis of the plaque size produced by the A-3/P16 hybrid DNA molecules revealed that the plaque morphology depended on the source of the *HindIII*-44% fragment; the small plaque morphology of P16 was observed for the P16 *HindIII*-44%/A-3 *HindIII*-56% hybrid while the large plaque morphology of A-3 was observed for the A-3 *HindIII*-44%/P16 *HindIII*-56% hybrid. The haemagglutination characteristics also segregate with the *HindIII*-44% fragment (Table 1) in a manner similar to plaque morphology.

To confirm that the hybrid DNAs actually contained the genetic information of the two different parental DNAs, plaques of the hybrid viruses were picked and virus stocks produced. The viral DNA derived from ³²P-labelled infected cells was digested with *HpaII* restriction endonuclease. Analysis of the fragments by gel electrophoresis (Fig. 2b) showed that the hybrid viruses constructed from the P16 and A-3 *HindIII* fragments exhibit *HpaII* cutting patterns expected for such hybrids. From the physical order

of polyoma restriction nuclease fragments⁷, it is known that the *HpaII*-3 and *HpaII*-5 are located in the *HindIII*-56% fragments whereas the portion of the *HpaII*-1 fragment containing the new *HpaII* cutting site of P16 is located in the *HindIII*-44% fragment. The small plaque hybrid virus constructed from the P16 *HindIII*-44% and A-3 *HindIII*-56% fragments contains *HpaII*-5 fragments which are smaller (migrate faster) than the equivalent P16 fragments and yet the hybrid contains the additional *HpaII* cutting site characteristic of P16. The large plaque hybrid virus constructed from the A-3 *HindIII*-44% and P16 *HindIII*-56% fragments contain *HpaII*-3 and *HpaII*-5 fragments which comigrate with the corresponding P16 *HpaII* fragments yet the hybrid does not contain the additional cutting site in *HpaII*-1 fragment which is characteristic of the P16 type.

Thus, the *HpaII* fragment patterns of the viruses confirm that hybrids between A-3 and P16 strains have been constructed and that they contain the sequences expected by our experimental approach. Furthermore, it is clear that the phenotypic difference between the P16 and A-3 strains of polyoma are not related to the genotypic differences located in the *HindIII*-56% fragment (that is the *HpaII*-3 and *HpaII*-5 fragment size differences). The genotypic difference which accounts for the phenotypic differences of the two strains must reside in the *HindIII*-44% fragment. Another plaque morphology variant and several late mutants have been mapped by a maker rescue technique in the *HpaII*-1 sequences contained in the *HindIII*-44% fragment (L.K.M. and M.F., manuscript in preparation). This region also contains the additional *HpaII* cutting site found in the P16 strain.

More exact determination of the locations of mutations affecting phenotype can be made by forming hybrid DNAs using different restriction nuclease fragments. Recently we have been able to construct hybrid molecules by ligation of restriction nuclease fragments produced by an enzyme from *H. haemolyticus* (*HhaI*). This enzyme produces fragments which contain a two-base overlap at their termini (Roberts and Myers, personal communication).

It has been possible to take advantage of the difference in restriction enzyme cleavage sites to analyse *in vivo* recombinants between adenovirus-2 ND₁ and adenovirus-5 (refs 8 and 9). We have used this type of genotypic analysis to characterise *in vitro* recombinants of polyoma. Construction of hybrid viruses *in vitro* provides a rapid and direct method for introducing genotypic markers into different virus strains.

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LOIS K. MILLER
MIKE FRIED

Department of Tumour Virus Genetics,
Imperial Cancer Research Fund,
PO Box 123, Lincoln's Inn Fields,
London WC2A 3PX, UK

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¹ Mertz, J. E., and Davis, R. W., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3370–3374 (1972).

- ² Ferguson, J., and Davis, R. W., *J. molec. Biol.*, **94**, 135-149 (1975).
³ Fried, M., Griffin, B. E., Lund, E., and Robberson, D. L., *Cold Spring Harb. Symp. quant. Biol.*, **39**, 45-52 (1974).
⁴ Crawford, L. V., *Virology*, **18**, 177-181 (1962).
⁵ Old, R., Murray, K., and Roizes, G., *J. molec. Biol.*, **92**, 331-342 (1975).
⁶ Thorne, H. V., Evans, J., and Warden, D., *Nature*, **219**, 728-730 (1968).
⁷ Griffin, B. E., Fried, M., and Cowie, A., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2077-2081 (1974).
⁸ Williams, J., Grodzicker, T., Sharp, P., and Sambrook, J., *Cell*, **4**, 113-119 (1975).
⁹ Sambrook, J., Williams, J., Sharp, P. A., and Grodzicker, T., *J. molec. Biol.*, **97**, 369-390 (1975).
¹⁰ Fried, M., *J. Virol.*, **13**, 939-946 (1974).
¹¹ Sharp, P. A., Sugden, B., and Sambrook, J., *Biochem.*, **12**, 3055-3063 (1973).
¹² Miller, L. K., and Wells, R. D., *J. biol. Chem.*, **247**, 2667-2674 (1972).

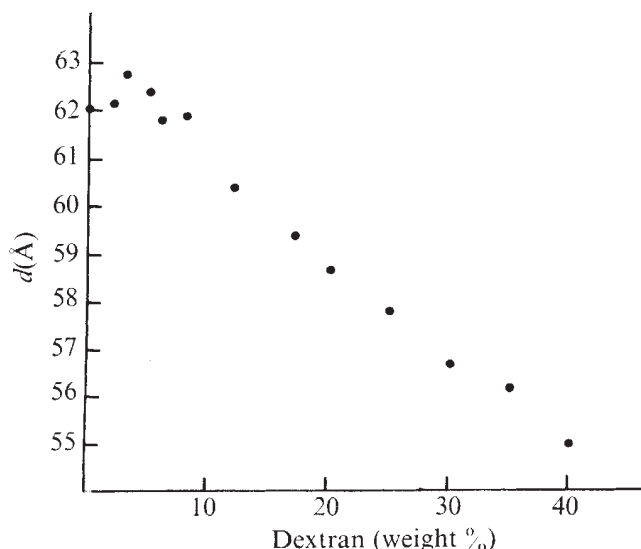
Measurement of forces between lecithin bilayers

CELL fusion¹ and vesicle-membrane² or vesicle-synapse³ fusion are currently thought to involve distance-dependent interactions between membranes. We have devised a general method for measuring such forces between planar phospholipid membranes, and report here results for the specific case of egg lecithin (phosphatidyl choline) bilayers in water.

When immersed in an excess amount of water, bilayers of zwitterionic lecithin form a lamellar lattice where the equilibrium bilayer separation is 27.5 Å (refs 4-7). We found that below this equilibrium spacing there is an apparent repulsion between bilayers, a repulsion that falls off exponentially with bilayer separation, with a decay distance of ~ 1.9 Å. The repulsive force for a separation < 22 Å is of the order of atmospheres, and reaches 10 atm by 17.5 Å. Our results indicate further that at the equilibrium spacing in pure water, repulsion is balanced by an attractive force between bilayers of $\sim 6.5 \times 10^4$ dyne cm⁻², or 0.065 atm.

To measure the net repulsion or 'disjoining pressure'⁸ we immerse pure egg lecithin into solutions of very high molecular weight dextran, rather than into pure water. The branched dextran molecules, having radii of gyration of the order of hundreds of Angstroms^{9,10}, apparently cannot enter the well defined lamellar structure which still forms. (Enclosing the lipid in dialysis membranes before exposure to dextran solutions has no observable effect on the lamellar phase.) At each higher dextran concentration, the lamellar phase must come to

Fig. 1 Lamellar repeat distance d as a function of dextran concentration. Chromatographically pure samples of egg lecithin (~ 30 mg) prepared by the method of Singleton *et al.*²⁰ were dispersed in 10 ml (10 g) of water or dextran (BDH: molecular weight 200,000-275,000) solution with the aid of sonication (30 s at half power on a Biosonic III). The lamellar lecithin phase was separated by ultracentrifugation. Scatter of points near 0% dextran is not systematic, but apparently arises from manipulation of the lipid. Repeat distances determined by X-ray diffraction are accurate to 0.1 Å.



equilibrium with water of lower chemical activity. Mechanically, the dextran solution exerts an osmotic pressure on the lamellar phase preventing full swelling of the lattice. At the new equilibrium, and for each dextran concentration, repulsion between bilayers is equal to the osmotic pressure of the dextran solution. The osmotic pressure is measured directly with a mercury manometer, or with pressure gauges. The resulting lattice spacings are measured by X-ray diffraction⁴⁻⁷. These spacings are shown in Fig. 1, where we plot the lattice repeat distance against dextran concentration.

To obtain a force-separation curve, we must calculate the spacing between leaflets at each dextran concentration. The total lattice repeat distance d is pictured as the sum of a biomolecular lipid layer of thickness d_l and a water layer of thickness d_w . To find d_l and d_w from d in Fig. 1, we use our X-ray diffraction data⁷ for repeat spacings d in the one phase lamellar lattice formed in $\lesssim 50\%$ water. The state of the one-phase system is defined by mixing set amounts of water and lipid (of volume fraction ϕ). When ϕ is set, one can say $d_l = \phi d$ and $d_w = (1 - \phi)d$. The lamellar repeat d increases monotonically with added water (until the limit of swelling where the second, pure water, phase begins to form). The same set of limited-water states may be reached by removing water osmotically from the fully swelled system. Specifically, one is observing the same thermodynamic state for lattices of a given d whether the lipid-water ratio has been determined by gravimetric means or osmotically. Then the d spacings of Fig. 1 can be converted to d_w and d_l thicknesses as described in the table. The data are shown as a force against separation curve in Fig. 2.

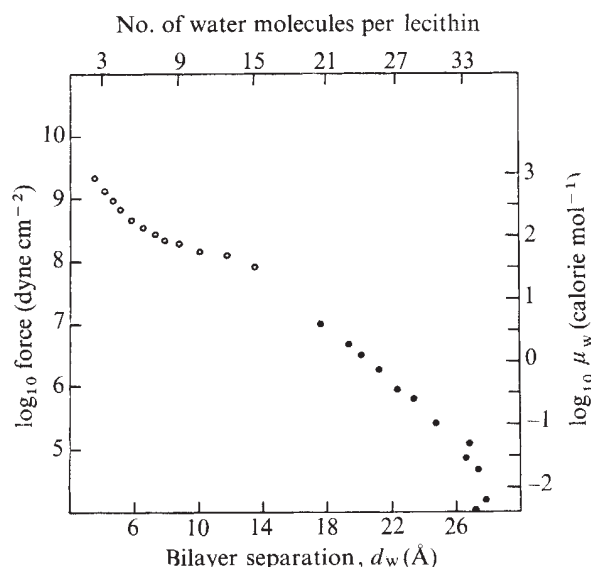


Fig. 2 Curve of force as a function of separation. Chemical potentials of water in the dextran solution (●) are found from $\mu_w = \text{osmotic force} \times \text{specific volume of water}$ (using present data for the osmotic force). Those from Elworthy's vapour pressure (○) are derived from $\mu_w = -RT \ln(p_w/p_w^{\text{sat}})$, assuming water density is not noticeably changed, and are measured in calorie mol⁻¹. Separation and water content per lecithin are as in Table 1. The relationship for pressure, $P_0 \exp(-d_w/\lambda)$, is convenient for discussing the osmotic data. Curve fitting to our osmotic pressure data (●) for $17.5 < d_w < 26.6$ Å gives $P_0 = 1.0 \times 10^{11}$ dyne cm⁻², $\lambda = 1.93$ Å.

The influence on d_l of any mono- or disaccharide breakdown products of dextran intercalated between bilayers is probably small. Besides the observed irrelevance of a dialysis membrane between dextran solution and lipid, we know that the replacement of water by glucose or sucrose solutions (of concentrations up to 40% by weight) has negligible effect on d , d_l and d_w when used in the one-phase lamellar system⁷.

The lipid thickness d_l increases with decreasing water content (Table 1). This increase in thickness itself indicates the repulsion

Table 1 Analysis of osmotic stress data

Weight per cent dextran	Osmotic pressure	d	d_l	ϕ	d_w	$\log_{10} F$ (dyne cm ⁻²)	$\log_{10} \mu_w$ (calorie mol ⁻¹)	Water- lecithin mol ratio
40		55.0	37.7	0.69	17.3			20.1
39.3	739				(17.5)	6.994	+0.627	
35		56.2	37.2	0.66	19.0			22.4
32.5	351				(19.3)	6.671	+0.304	
30		56.7	37.1	0.65	19.6			23.2
28.5	243				(20.1)	6.511	+0.144	
25	141	57.8	36.6	0.636	21.2	6.268	-0.092	25.4
20	65.8	58.7	36.3	0.62	22.4	5.943	-0.42	27.1
17	43.8	59.4	36.0	0.61	23.4	5.766	-0.60	28.5
12	19.5	60.4	35.6	0.59	24.8	5.415	-0.95	30.6
8	9.1	61.9	35.1	0.57	26.8	5.082	-1.28	33.5
6	5.4	61.8	35.2	0.57	26.6	4.853	-1.51	33.2
5	3.6	62.4	35.0	0.56	27.4	4.681	-1.69	34.4
3	1.2	62.8	34.9	0.56	27.9	4.204	-2.16	35.1
2	0.8	62.2	35.0	0.56	27.2	4.028	-2.34	34.1
0	0	62.1	35.1	0.57	27.0			33.7

Osmotic pressures of the dextran solutions were made directly using a mercury manometer or pressure gauge. Solutions connected to the manometer were in contact with distilled water by way of a dialysis membrane. Putative volume fractions of lipid, ϕ , in the lamellar phase were found by matching against separations seen in the single phase formed at low total water content⁷. Separation d_w is then $(1-\phi)d$, where the lamellar repeat distance d is measured by X-ray diffraction. Values in parentheses are interpolated between adjacent measured values. The definition of d as a separate layer containing only water is arbitrary. The aqueous region could also be defined to include the lipid polar moiety⁶. In that case the apparent thickness d will be greater than the values derived here. It is also a matter of convenience to picture the lipid-water interface as a sharp step where it is actually an interface of thickness comparable to the dimensions of the polar groups.

between bilayers¹¹, which is being quantitatively measured here.

At a separation of 27.5 ± 0.5 Å, the lamellar lattice is in thermodynamic equilibrium with pure water. Because of its rapid exponential variation, the repulsive component apparently dominates the net interaction for separations only a fraction of an Angstrom less than the equilibrium separation in water. By taking the repulsion curve $P_0 \exp(-d_w/1.9 \text{ Å})$ derived from fitting the experimental points of Fig. 2 that lie in the range $17.5 < d_w < 26.6$ Å, and by extrapolating to the equilibrium separation of 27.5 Å in pure water, we estimate the repulsive part of the force between bilayers at equilibrium in water. Since this repulsion is equal and opposite to bilayer attraction, this method provides a first estimate of the interlamellar attraction force, 5×10^4 – 8.5×10^4 dyne cm⁻². The error, which appears among different lipid preparations, and probably results from handling the lipid, here assumes an ambiguity of 0.5 Å in the determination of the 27.5 Å equilibrium spacing.

Our results using osmotic stress on the lamellar lattice are supplemented by those of Elworthy¹² (supplied to us in tabular form) who determined the amount of water imbibed by egg lecithin as a function of aqueous vapour pressure, p_w . The chemical potential, $\mu_w = -RT \ln(p_w/p_w^{\text{sat}})$, measures the work of transferring water from the lamellar lattice vapour pressure p_w to bulk water (vapour pressure p_w^{sat}). By dividing the work per mol by the molar volume (the value for pure bulk water is used here), the force per area between the bilayers from which water is removed is obtained. Lamellar repeat distances and bilayer separations are known from X-ray diffraction on lecithin⁷ at these limited amounts of water. These vapour pressure measurements are plotted as the open circles in Fig. 2. (The strict similarity of the egg lecithin used in the imbibing and X-ray experiments is not established. The temperature in both studies was 25 °C, but was not strictly controlled in our case.)

The curve of force as a function of separation in Fig. 2 suggests that the vapour pressure and osmotic pressure experiments are consistent. But comparison is difficult because there is a 4 Å gap in data (between 13.5 and 17.5 Å separation). To this extent there does not seem to be a sharp jump in the work of removal of water at any point in the curve from 3 to 33 molecules of water per lecithin; within the limits of these measurements, there is a smooth change in the work of removing water near the lecithin polar groups. It is clear that the simple exponential law (solid points, osmotic data) is not maintained

when more than two-thirds of the original water is removed from the lamellar lattice.

Analyses of the amount of water near lecithins using thermal¹³ and resonant¹⁴ probes reveal 10–12 'hydrating' or 'bound' water molecules per lecithin molecule, with another 11 waters 'trapped' by each lecithin¹⁴. These probes would suggest that the remaining water is bound loosely enough to be considered bulk water. But the finite work of removal, μ_w , which we measure osmotically shows this is not so.

The repulsion between lecithin bilayers probably arises both from electrostatic interaction between charged groups and from energy of hydration of all polar groups on the molecule. In the presence of solvent boundaries, as occur here between lipid and water, the change in hydration might be visualised in terms of electrostatic image forces. Between molecules without charged groups, repulsion would occur because of hydration energies alone. The exponential force measured here (Fig. 2) does not fit a model¹⁷ where the lecithin polar group is thought to form an internal double layer.

The attractive force is of the order of magnitude expected for van der Waals' forces in lipid-water multilayers¹⁸. Its identity with these forces remains to be established by determination of the attractive force as a function of separation. This we are doing by applying osmotic stress to lamellae containing varied fractions of charged lipids in salt water media. We measure double-layer repulsions which will increase the equilibrium separation^{7,19}. From each lamellar repulsion curve we may infer an attractive force at each equilibrium separation.

The work of pushing together two electrically neutral zwitterionic phospholipid bilayers can be significant compared with energies available from thermal motion in collision. To bring two faces of area 100 Å² to even 15 Å separation would require about 8×10^{-13} erg or 20 kT units. Alternatively, using the Derjaguin step approximation^{15,16} to convert the interaction of two parallel planes to one between a planar bilayer and a spherical bilayer vesicle, a sphere of outermost radius 300 Å coming within 14 Å of contact also feels a barrier of about 20 kT . Such large energies suggest that when fusion of two pure lipid vesicles is in fact observed experimentally, there might be some destabilisation or transient rearrangement of the phospholipid molecules to push apart the polar head groups. This would allow the hydrocarbon moieties to merge without requiring a large amount of work to remove water from the polar groups as observed here for uniform planar bilayers.

We hope that coupled energetic and structural measurements,

such as we report here, will lead to more precise analyses of the forces responsible for lamellar stability.

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D. M. LeNEVEU
R. P. RAND

Brock University,
St Catharines,
Ontario, Canada

V. A. PARSESIAN

National Institutes of Health,
Physical Sciences Laboratory,
Division of Computer Research and Technology,
Bethesda, Maryland 20014

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- ¹ Ahkong, Q. F., Fisher, D., Tampion, W., and Lucy, J. A., *Nature*, **253**, 194–195 (1975).
- ² Pagano, R., Huang, L., and Wey, C., *Nature*, **252**, 166–167 (1974).
- ³ Muller, R., and Finkelstein, A., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 923–926 (1974).
- ⁴ Small, D. M., and Bourges, M., *Molec. Cryst.*, **1**, 541 (1966).
- ⁵ Reiss-Husson, F., *J. molec. Biol.*, **25**, 363–382 (1967).
- ⁶ Small, D. M., *J. Lipid Res.*, **8**, 551–557 (1967).
- ⁷ LeNeveu, D. M., thesis, Brock Univ. Ontario, (1973).
- ⁸ Derjaguin, B. V., Voropayeva, T. N., Kabanov, B. N., and Titiyevskaya, A. S., *J. Colloid Sci.*, **19**, 113–135 (1964).
- ⁹ Senti, F. R., et al., *J. Polym. Sci.*, **17**, 527–546 (1955).
- ¹⁰ Granath, K. A., *J. Colloid Sci.*, **13**, 308–328 (1958).
- ¹¹ Parsegian, V. A., *J. theor. Biol.*, **15**, 70–74 (1967).
- ¹² Elworthy, P. H., *J. chem. Soc.*, 5385–5389 (1961); *ibid.*, 4897–4900 (1962).
- ¹³ Chapman, D., Williams, R. M., and Ladbroke, B. D., *Chem. Phys. Lipids*, **1**, 445–475 (1967).
- ¹⁴ Finer, E. G., and Darke, A., *Chem. Phys. Lipids*, **12**, 1–16 (1974).
- ¹⁵ Derjaguin, D. V., *Kolloid-Zeit.*, **69**, 155–164 (1935).
- ¹⁶ Verwey, E. J. W., and Overbeek, J. Th. G., in *Theory of the Stability of Lyophobic Colloids*, ch. 9 (Elsevier, Amsterdam, 1948).
- ¹⁷ Parsegian, V. A., *Science*, **156**, 939–942 (1967).
- ¹⁸ Ninham, B. W., and Parsegian, V. A., *J. chem. Phys.*, **53**, 3398–3402 (1970).
- ¹⁹ Gulik-Kryzwicki, T., Tardieu, A., and Luzzati, V., *Molec. Cryst. liq. Cryst.*, **8**, 285–291 (1969).
- ²⁰ Singleton, W. S., Gray, N. S., Brown, M. L., and White, J. L., *J. Am. Oil Chem. Soc.*, **42**, 53–56 (1965).

Stimulation and suppression of response of mouse T cells to the schistosomules of *Schistosoma mansoni* during infection

INFECTION of mice with cercariae of *Schistosoma mansoni* produces a disease with immunopathology similar to human bilharziasis¹, the chronic hepatosplenomegaly being probably secondary to granulomatous reactions to eggs deposited in the tissues². It has been shown that thymus-derived lymphocytes have a major role in the granulomatous reaction^{3,4}, but the reaction of the T cells to earlier stages of the parasite has not hitherto been studied. We have investigated one such reaction—the helper T-cell response to the schistosomule—by using schistosomules as carriers for a hapten and measuring the anti-hapten response in normal and infected mice. In analogous experiments using protein carriers, the anti-hapten response has been shown to be due to cooperation between T cells and B cells⁵. We have found evidence for a specific T-dependent helper response which is greatly increased during the first 2 weeks after infection and subsequently diminishes, and we suggest that the diminution may partly represent a specific suppression of helper T-cell function.

(C57BL × BALB/c)F₁ mice and *nu/nu* (athymic) mice of various genotypes were infected with 40 cercariae of *S. mansoni* on the shaved abdomen⁶. Schistosomules were prepared *in vitro* by mechanical agitation to cercariae in a Vortex mixer⁷, isolation of the cercarial bodies by layering the mixture of bodies and tails on to the Hanks' buffered salts solution (HBSS) in a test tube, where the bodies sink to the bottom in 10 min, and incubation of this pellet for 24 h at 37 °C in RPMI-1640 with 20 mM HEPES in atmosphere of 5% CO₂ in air. The resulting schistosomule preparations were con-

Table 1 Carrier effect of schistosomules in the antibody response of normal mice to TNP

Mice	No. of schistosomules injected intravenously	TNBS used for coating (mg/ml)	No. of mice	4 d anti-TNP PFC per spleen (geometric mean ± s.e.)
Normal	—	—	6	116(92–147)
	100	—	9	260(200–338)
	100	0.5	3	980(922–1,040)
	100	1.0	4	603(443–820)
	100	1.4	3	2,996(2,287–3,926)
	100	2.0	3	1,395(1,186–1,644)
	100	5.0	13	1,765(1,505–2,070)
	100	14.0	3	3,737(3,350–4,169)
	300	5.0	3	6,269(5,596–7,025)
	900	5.0	3	26,037(20,672–32,796)
	2500	5.0	7	31,466(22,403–44,196)
	2000	1.4	3	20,527(19,204–21,942)
Nude	2000	14.0	3	28,639(21,061–38,942)
	100	5.0	3	840(804–877)
	2000	5.0	5	4,755(3,750–6,748)

taminated with less than 5% cercarial tails. For vaccination, schistosomules were fixed in 0.06% formaldehyde for 5 min, followed by three washings in HBSS. For use as carriers for the hapten, schistosomules were coated with trinitrophenol (TNP) by a modification of the method of Rittenberg and Pratt⁸: 5,000–10,000 schistosomules were incubated in a 0.5% solution of trinitrobenzene-sulphonic acid (TNBS) in 0.28 M cacodylate buffer (pH 6.9), for 30 min, at 37 °C, then washed with modified barbital buffer (pH 7.1), followed by three washes in HBSS. TNP-coated schistosomules were injected into mice and the direct anti-TNP plaque forming cell (PFC) responses in their spleens were measured at various intervals thereafter, using lightly TNP-coated sheep erythrocytes⁹, by the slide method¹⁰. Preliminary experiments showed that intravenous injection produced a better response than intraperitoneal and that the response was maximal if assayed 4 d after infection. This procedure was adopted accordingly for experiments on groups of normal, nude, infected or vaccinated mice. For specificity controls, other groups of infected mice were injected intravenously with TNP-coated horse erythrocytes and the TNP response measured as above.

In normal mice, schistosomules showed a weak carrier effect, at least 900 TNP-schistosomules being needed to give a substantial anti-TNP response. The use of higher or lower TNBS concentrations for coating schistosomules did not greatly affect the result. Nude mice responded very much less well suggesting that the carrier effect was largely T-cell mediated (Table 1).

In mice infected with 40 cercariae, as little as 100 TNP-schistosomules produced a massive anti-TNP response, greatest when the TNP-schistosomules were injected 10 d after the

Table 2 Failure of carrier effect to increase following a second infection with *S. mansoni* cercariae

	Anti-TNP PFCs per spleen (geometric mean ± s.e.) (Days after infection)		
	0	7	10
First infection (40 cercariae)	1,765 (1,505–2,070)	88,495 (80,433–97,366)	233,675 (218,488–249,917)
Second infection at 5 weeks (40 cercariae)	25,098 (20,651–30,503)	20,973 (14,760–29,801)	38,193 (33,650–43,348)
Second infection at 10 weeks (40 cercariae)	4,971 (3,522–7,016)	5,783 (5,389–6,205)	11,181 (9,400–13,300)

(C57BL × BALB/c)F₁ mice were infected with 40 cercariae either once or twice at 5- or 10-week intervals, and injected with 100 TNP-schistosomules 7 or 10 d later.

infection, and thereafter declining, but only falling to the level of response in non-infected mice at about week 12, or 13 when the mice were beginning to die. This increased response to TNP-schistosomes was significantly lower in nude mice, showing that the increase predominantly represents a response of T-helper cells. Mice at all but the latest stages of infection responded normally to TNP-horse red blood cells, showing that the increased T-cell response was immunologically specific (Fig. 1). Similar results have been obtained in CBA mice. In mice infected with *S. mansoni* but not injected with TNP-schistosomes, we noted that the background PFC against both TNP and SRBC began to rise from 4 weeks, possibly an indication of nonspecific B-cell activation.

In mice vaccinated with 40 formalin-fixed schistosomes a substantial but smaller helper T-cell response was seen, which was maintained for at least 13 weeks after vaccination. This suggests that both the early increase and the subsequent decline may be partly due to the living infection (Fig. 2).

In view of the fall in the helper T-cell response in the later stages of the infection (see Fig. 1), mice that had been infected 5 or 10 weeks earlier were reinfected with 40 cercariae and tested with TNP-schistosomes 7 or 10 d later. At no stage was there a significant increase in the TNP-response (Table 2). In other words, the helper T-cell response to the second infection was significantly lower than that to the first, indicating that the decline in helper T-cell activity was not due merely to the disappearance of antigens restricted to the schistosome. We hope to establish by serum and cell-transfer experiments whether this decline in the T-cell response is caused by antibody and/or antigen, as has been shown in tumour-bearing animals¹¹ or perhaps by specific suppressor cells. One possibility is that this T-cell tolerance is associated with the deposition of eggs,

Fig. 1 Increased carrier effect of schistosomes following infection with *S. mansoni* cercariae. The anti-TNP PFC response was measured in the spleens of mice infected with 40 *S. mansoni* cercariae and subsequently injected with 100 TNP-schistosomes or 2×10^8 TNP-horse RBCs. The responses were assayed 4 d later, but are plotted against the day of injection. ●, (C57BL $\times$ BALB/c)F₁ mice injected with 100 TNP-schistosomes; * nude mice injected with 100 TNP-schistosomes; ○ (C57BL $\times$ BALB/c)F₁ mice injected with 2×10^8 TNP-horse RBCs. The zero time point represents the response to 100 TNP-schistosomes in non-infected mice.

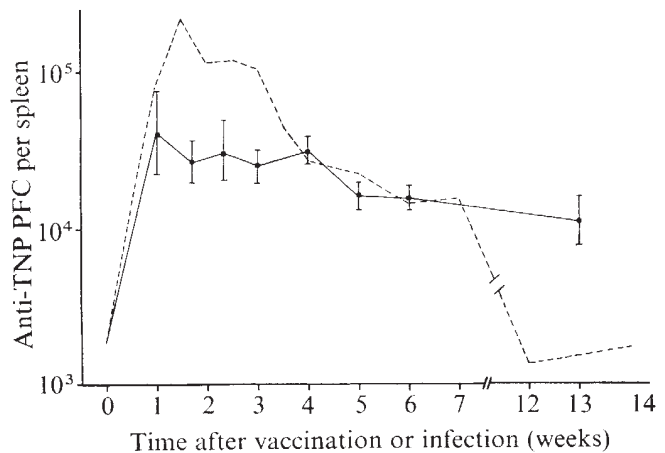
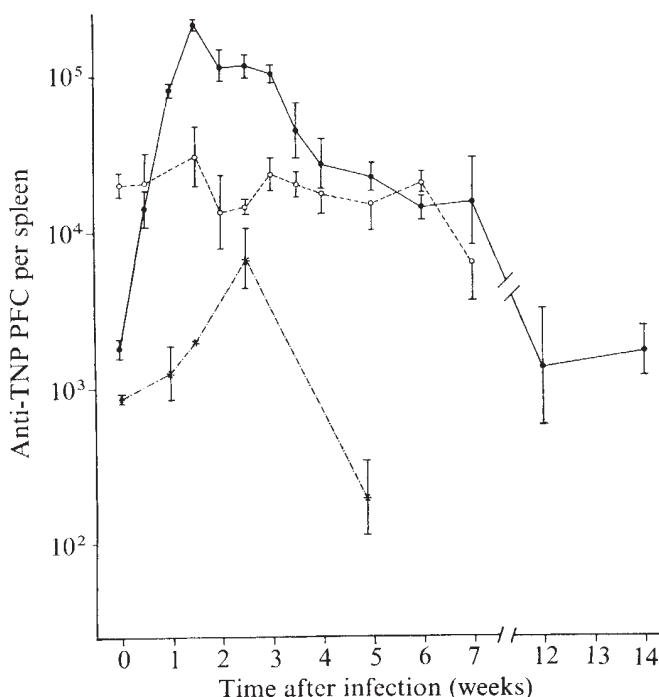


Fig. 2 Increased carrier effect of schistosomes following vaccination with dead schistosomes. The anti-TNP PFC response was measured in the spleens of (C57BL $\times$ BALB/c)F₁ mice vaccinated with 40 formalin-fixed schistosomes and subsequently injected with 100 TNP-schistosomes. The broken line indicates the response of infected mice (see Fig. 1).

which release antigens known to strongly stimulate T cells³. Egg-laying does not however begin until about 5–6 weeks after cercarial penetration, whereas the reduction in helper T-cell responsiveness is already apparent by 4 weeks (Fig. 1). On the other hand, the granulomatous response to eggs in the tissues, which is also a T-cell mediated response^{2,3}, also decreases in the course of time^{1,13}, and it is tempting to speculate that the underlying mechanisms may be similar: in the case of our helper T cells, circulating antigens derived from the adult worm but shared with the schistosome may be responsible for the reduced T-cell help late in the infection.

It is interesting that protective immunity in mice infected with *S. mansoni* reaches a peak relatively late (approximately 12 weeks) after infection¹⁴, at a time when the helper T-cell response has declined to the level in uninfected mice (Fig. 1). Protection seems to be at least partly dependent on antibody¹⁵, which suggests that other factors than T-cell reactivity may be responsible for its unexpectedly late development. It would clearly be of great value to be able to follow the response of the antibody-forming (B) cells during infection, with the same degree of precision as is now available for the T cells.

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F. J. RAMALHO-PINTO
J. B. DE SOUZA
J. H. L. PLAYFAIR

Department of Immunology,
Middlesex Hospital Medical School,
London W1, UK

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- 1 De Witt, W. B., and Warren, K. S., *Am. J. trop. Med. Hyg.*, **8**, 440–446 (1959).
- 2 Warren, K. S., *Trans. R. Soc. trop. Med. Hyg.*, **66**, 417–432 (1972).
- 3 Domingo, E. O., and Warren, K. S., *Am. J. Pathol.*, **51**, 757–767 (1967).
- 4 Fine, D. P., Buchanan, R. D., and Colley, D. G., *Am. J. Pathol.*, **71**, 193–206 (1973).
- 5 Mitchison, N. A., *Eur. J. Immun.*, **1**, 18–27 (1971).
- 6 Smithers, S. R., and Terry, R. J., *Parasitology*, **55**, 695–700 (1965).
- 7 Ramalho-Pinto, F. J., et al., *Expl Parasit.*, **36**, 360–372 (1974).
- 8 Rittenberg, M. B., and Pratt, K. L., *Proc. Soc. exp. Biol. Med.*, **132**, 575–581 (1969).
- 9 Kettman, J., and Dutton, R. W., *J. Immun.*, **104**, 1558–1561 (1971).
- 10 Cunningham, A. J., and Szenberg, A., *Immunology*, **14**, 599–601 (1968).
- 11 Gorczynski, R. M., et al., *Nature*, **254**, 141–143 (1975).
- 12 Andrade, Z. A., and Warren, K. S., *Trans. R. Soc. trop. Med. Hyg.*, **58**, 53–57 (1964).
- 13 Colley, D., *J. Immun.*, **115**, 150–156 (1975).
- 14 Sher, A., Mackenzie, P., and Smithers, S. R., *J. infect. Dis.*, **130**, 626–633 (1974).
- 15 Sher, A., Smithers, S. R., and Mackenzie, P., *Parasitology*, **70**, 347–357 (1975).

matters arising

Similarities between "cholinergic proteolipid" and detergent-extracted cholinergic proteins

A REPORT from Changeux's group¹ concludes that the cholinergic receptor proteolipid² is different from the detergent-extracted cholinergic protein³. This conclusion is based on the observation that there is no immunological cross reactivity between the proteins and their respective antisera. Furthermore, confirming some earlier observations of Karlin⁴, they found that after affinity labelling of the receptor *in situ* with ³H-MPTA, the radioactive ligand is not extracted by chloroform-methanol. They failed, however, to establish whether the cholinergic receptor proteolipid is still extractable after the affinity labelling and thus committed the same error as did Karlin previously.

In an attempt to clarify the problem we have repeated the experiments (Table 1). In membranes from *Electrophorus* we found that treatment with dithiothreitol (the preliminary step in the affinity labelling) followed by mercaptoethanol, produces a drastic reduction (61%) in the receptor proteolipid that can be extracted. By affinity labelling *Torpedo* membranes with ³H-MPTA we confirmed the findings of Karlin⁴ and Changeux¹ in the sense that only 6% and 14% of the radioactivity was extracted with chloroform-methanol; however in the same experiments there was a reduction of 70% and 80% in the cholinergic receptor proteolipid (Table 1). Thus an alternative and more valid conclusion to draw from the experiments of both Changeux¹ and Karlin⁴ may be that the cholinergic receptor proteolipid is labelled in the membrane but is no longer extractable by the organic

solvents, although it can still be solubilised with strong detergents in aqueous solutions.

With respect to the immunological work, we can offer no explanation at present for the apparently negative results. The isolation procedure used by Changeux's group, however, is not that used by our group³. Thus the diethyl ether precipitate used by them contains only 10% of the total proteolipids and practically no cholinergic receptor proteolipid. Clearly therefore the question of the immunological comparison of the proteins must be investigated further before definite conclusions can be drawn.

It is convenient to summarise the many similarities between these two proteins. Both extraction procedures are designed to solubilise hydrophobic intrinsic membrane proteins and both proteins have been purified by affinity chromatography either in aqueous solutions⁵ or in organic solvents⁶.

The number of receptor sites that can be labelled with α -toxin in *Electrophorus*¹ and *Torpedo* corresponds closely to the amount of cholinergic receptor proteolipid that we extract⁷. Both isolated proteins show high affinity binding for a range of cholinergic nicotinic ligands, including acetylcholine, decamethonium, *d*-tubocurarine and α -bungarotoxin. We can transfer our proteolipid to aqueous solutions using Triton X-100 and in these conditions the binding of ³H- α -bungarotoxin can be demonstrated. Incorporation of the proteolipid into lecithin liposomes has also been achieved and the binding of α -toxin in the aqueous solution has been observed. From this evidence we conclude that the cholinergic proteolipid of the electroplax is probably identical with the binding subunit of the detergent-extracted protein.

We thank Dr J. P. Changeux for ³H-MPTA and *Torpedo* tissue and Dr G. G. Lunt for helpful discussion.

E. DE ROBERTIS
SARA FISZER DE PLAZAS
MARIA C. L. DE CARLIN

Instituto de Biología Celular,
Facultad de Medicina,
Universidad de Buenos Aires,
Argentina

- 1 Barrantes, F. J., Changeux, J. P., Lunt, G. G., and Sobel, A., *Nature*, **256**, 325-327 (1975).
- 2 La Torre, J. L., Lunt, G. S., and De Robertis, E., *Proc. natn. Acad. Sci. U.S.A.*, **65**, 716-720 (1970).
- 3 Changeux, J. P., Kasai, M., and Lee, C. Y., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1241-1247 (1970).
- 4 Karlin, A., *Life Sci.*, **14**, 1385-1415 (1974).
- 5 Meunier, J. C., Sealock, R., Olsen, R., and Changeux, J. P., *Eur. J. Biochem.*, **45**, 371-394 (1974).
- 6 Barrantes, F. J., Arbilla, S., de Carlin, M. C. L., and De Robertis, E., *Biochem. biophys. Res. Commun.*, **63**, 194-201 (1975).
- 7 Fiszer de Plazas, S., and De Robertis, E., *Biochim. biophys. Acta*, **274**, 258-265 (1972).

BARRANTES ET AL. REPLY—As De Robertis *et al.* point out¹, the extent to which chloroform-methanol (2:1) (C:M) extracts proteolipids after treatment of the receptor-rich membranes with dithiothreitol (DDT) and reaction with ³H-MPTA was not mentioned in our report in *Nature*². It was, however, a daily experience in the laboratory that such treatments did not modify, in any respect, the dissolution of the receptor protein by detergents such as Triton X-100 or sodium cholate; but as De Robertis claims, this might no longer be true in the case of the extraction of the proteolipids by chloroform-methanol.

To our surprise, when De Robertis carries out this control he assays the proteolipid by measuring ¹⁴C-acetylcholine binding after affinity labelling with ³H-MPTA. From Karlin's work and from our own experience it is well established that ³H-MPTA attaches covalently to the cholinergic receptor site and therefore blocks cholinergic ligand binding. On the other hand, the fact that acetylcholine binds to the proteolipid after reaction with ³H-MPTA suggests that, in the presence of organic solvents, ¹⁴C-acetylcholine binding is largely nonspecific (and this might equally be true for α -bungarotoxin binding in such drastic conditions). Also C:M extracts about the same fraction (0.5-1%) of the total proteins from both fresh

Table 1 Percentage reduction in the extraction of the cholinergic proteolipid from membranes of *Electrophorus* and *Torpedo* electroplax, after affinity labelling with ³H-MPTA

	μ g Receptor proteolipid per g fresh tissue		Reduction (%)
	Control	Treated	
<i>Electrophorus</i> *	5.2	2.0	61
<i>Torpedo</i> †	23.0	7.0	70
<i>Torpedo</i> †	24.9	5.0	80

The experiments were carried out as described in ref. 1. The amount of receptor proteolipid was determined after chromatography of the extract on Sephadex LH-20 in the presence of 10^{-6} M ¹⁴C-acetylcholine².

*Treated with dithiothreitol and mercaptoethanol.

†Affinity labelled with ³H-MPTA (4-(*N*-maleimido)-phenyltri-³H-methylammonium).

Torpedo electric tissue and purified membrane fragments enriched 50–100 times in α -toxin sites and containing the cholinergic receptor as 10–30% of their protein.

Nevertheless, we were able to repeat the observation made by De Robertis when the proteolipid was measured by the method of Lowry as modified by Hess and Lewin. Starting from 1 ml of a suspension of membrane fragments of specific activity 890 nmol of toxin sites per g of protein (6.6 mg protein per ml) the C:M extract contained 17 μ g protein after MPTA labelling as compared with 76 μ g protein in the absence of DTT and MPTA.

If after ^3H -MPTA labelling, however, the membrane fragments are exposed to an oxidising reagent such as 1 mM 5,5'-diithiobis-(2-nitrobenzoic acid) (DTNB) C:M extracts 46 μ g of protein from 1 ml of membrane suspension, a value closer to that found before DTT treatment. In both conditions, that is after ^3H -MPTA labelling and with or without DTNB treatment less than 2% of ^3H -MPTA (and not 6% and 14% as mentioned by De Robertis) is extracted into the C:M. Therefore, -SH reagents markedly modify the extractability of the proteolipids by C:M but show no effect on that of the radioactively labelled receptor.

During these control experiments, we noticed that DTT added to the C:M extract of proteolipid considerably enhances its transfer into an aqueous 1% Triton X-100 phase (the ratio of aqueous to organic phase of 2.6:13.0 becomes 16.0:1.6 μ g protein per ml in the presence of 1 mM DTT). Nevertheless, the DTT-solubilised proteolipids extracted by Triton X-100 still do not bind ^3H -toxin from *N. nigricollis* in the presence of physiological Ringer solution in the conditions routinely used for receptor assay.

Finally, a comparison has been made between native receptor-rich membrane fragments and membranes which have been extracted with C:M. SDS-polyacrylamide electrophoretograms of both preparations reveal the presence of the 39,000–41,000 daltons band characteristic of the cholinergic receptor protein. Electrophoretograms of membranes labelled with ^3H -MPTA show this to be the only band to be radioactively labelled and confirm its identity as the receptor.

These observations give additional support to one of our initial conclusions; that the C:M-extracted cholinergic proteolipid shows little if any relationship to the now well identified cholinergic receptor protein in its detergent-extracted form.

Climate in the 1970s

A FEW words near the end of our letter¹ headed "Climatic reversal in northern North Atlantic", seem to have resulted in our diagnosis of recent climatic shifts being so widely misrepresented elsewhere that some further statement is necessary.

Our letter gave a diagnosis of what had been happening in the North Atlantic-European sector between 1971 and 1975, and was explicitly not a forecast. That diagnosis was, and is, valid, though it was probably not advisable to use the words "little ice age" to describe two decades of colder climate, and it was certainly not advisable to suggest that that episode was over just because of a run of four or five mild winters in Europe, when the other seasons (in spite of August 1975!) continue mainly cold, and the generally lower level of temperatures over the Arctic above 70°N, which set in about 1961, persists.

What has happened since 1971 may be largely attributed to a change of the prevailing wave positions in the circumpolar vortex, even though we do not yet know what causes such shifts involving the centre of the polar cap itself. In 1971 the Canadian Arctic became, for the first time, involved in the cooling that set in sharply in other sectors of the far north in 1961, and, just as sharply, the coldest centre was transferred to the Canadian sector. This meant that, there was a great increase in the south to north thermal gradient between the western Atlantic and northern Canada, particularly in the winter months, resulting in a great increase in the energy of the atmospheric circulation over the North Atlantic, driving mild air towards Europe and more saline water to Iceland—as M. Rodewald (unpublished) has pointed out. But these conditions do not seem to have penetrated as far into the Arctic as during the general Arctic warming in the 1920s and 1930s, and it is not yet clear whether the mechanism described since 1971 is capable of restoring conditions there to what they were before 1960. Doubts on this are strengthened by observation that the centre of the polar cold regime and the waves in the circumpolar vortex returned to their 1960s' positions for about six months around late 1973 and again in 1975. The ice then again increased in the sector east of Greenland, and once more approached the coasts of Iceland in July and August 1975, perhaps for the first time this century in those months.

S.-A. Malmberg informs me that in June 1975 off north Iceland the salinity anomaly was slight, only -0.03‰ , though the temperature anomaly was -1.60 °C . No influx of the Atlantic watermass into northeast Icelandic waters was observed in June 1975. The ice conditions were "relatively unfavourable", and by July 1975 there was ice in the East Iceland Current. In June 1975 both the polar and

Atlantic currents were weak in the north Iceland region. Sometimes both these currents are strong (as in 1964 and 1968), and sometimes one is strong and the other weak: no relationship has been found between them.

Recent investigations^{2–4} imply that the great variations to which the volume of polar water transported by the East Greenland Current and the southward penetration of this water in the East Iceland Current are prone, may be one of the most interesting and important aspects of climatic change, affecting alike the development of major ice ages, the little ice age of recent centuries, and the cooling since the 1950s.

H. H. LAMB

School of Environmental Sciences,
University of East Anglia

¹ Dickson, R. R., Lamb, H. H., Malmberg, S.-A., and Colebrook, J. M., *Nature*, **256**, 479–481 (1975).

² McIntyre, A., Ruddiman, W. F., and Jantzen, R., *Deep-Sea Res.*, **19**, 61–77 (1972).

³ McIntyre, A., *Climatic Research Unit Research Publication No. 2* (CRU RP 2), 41–47 (University of East Anglia, Norwich, 1974).

⁴ Lamb, H. H., *Proc. WMO/IAMAP Symposium on Long-term climatic fluctuations, Norwich, August 18–23, 1975*, 473–477 (WMO, Geneva, 1975).

Lattice absorption in small particles

LUKES¹ has suggested that the width of the lattice absorption peak for small particles of ionic solids at very low temperatures would be dominated by collisions of phonons with the surfaces. The estimate given for the phonon collision time, $\tau \sim R/v$, where R is the particle radius and v is the velocity of sound in the bulk, is, however, applicable to acoustic phonons only. This collision time is not relevant to the phenomenon of lattice absorption, which is due to the long-wave optical phonons. The collision time τ enters the calculations of lattice thermal conductivities at very low temperatures, when all other possible scattering mechanisms become ineffective. This is a well known boundary effect which has been investigated extensively^{2,3}.

As regards lattice absorption by small particles, many experiments have shown the absorption peak to be considerably broader than that calculated by using the bulk value of the damping constant γ appearing in the expression for the dielectric constant^{4,5}. For small NaCl and MgO crystallites ($R \lesssim 1\text{ }\mu\text{m}$) it has been estimated⁶ that the damping parameter increases beyond its bulk value by a factor of ~ 2.5 . No fundamental calculations of γ in small particles, analogous to those which exist for bulk crystals⁷, have so far been performed.

R. RUPPIN

Soreq Nuclear Research Centre,
Yavne, Israel

¹ Lukes, T., *Nature*, **255**, 623 (1975).

² Casimir, H. B. G., *Physica*, **5**, 495–500 (1938).

³ Carruthers, P., *Rev. Mod. Phys.*, **33**, 92–138 (1961).

⁴ Genzel, L., and Martin, T. P., *Phys. Status Solidi*, **(b)**, **51**, 91–99 (1972).

⁵ Ruppin, R., *Surf. Sci.*, **34**, 20–32 (1973).

⁶ Fuchs, R., *Phys. Rev.*, **B**, **11**, 1732–1740 (1975).

⁷ Ipatova, I. P., Maradudin, A. A., and Wallis, R. F., *Phys. Rev.*, **155**, 882–895 (1967).

¹ De Robertis, E., de Plazas, S. F., and de Carlin, M. C. L., *Nature*, **259**, 605 (1976).

² Barrantes, F. J., Changeux, J. P., Lunt, G. G., and Sobel, A., *Nature*, **256**, 325–327 (1975).

reviews

Landmarks in North Sea geology

Petroleum and the Continental Shelf of North-West Europe. Volume 1: Geology. Edited by Austin W. Woodland. Pp. ix+501. (Applied Science Publishers: Barking, UK, 1975; published on behalf of the Institute of Petroleum.) £16.

THIS book is outstanding, and a credit to all the people and organisations who were responsible for its production. In November 1974 a conference was held at the Bloomsbury Centre, London, organised by the Institute of Petroleum, the Petroleum Exploration Society of Great Britain, the UK Offshore Operators' Association, the Geological Society of London, and the Institute of Geological Sciences. This meeting was attended by some 900 geologists from oil companies, universities and state organisations.

The first three days were devoted to the geology of offshore Britain, especially the North Sea. The last two days were devoted to environmental problems associated with subsea petroleum production. This volume contains the 38 papers presented in the first three days. Broadly speaking, the papers fall into three main groups: geophysical surveys of north-western and south-western British waters, as yet largely unexplored; a series of papers giving broad and system-by-system reviews of North Sea stratigraphy and sedimentation; a third group of papers are field case histories. These document most of the southern gas area fields, and a fair number of the Jurassic, Chalk and Tertiary oilfields of the northern North Sea. Coverage is not just confined to British waters, but includes material ranging geographically from seismic sections off the northern Norwegian coast, through descriptions of oil fields in Norwegian and Danish waters (Ekofisk and Dan fields) to gas fields in onshore Holland.

There is a comparable breadth of technical information. This ranges from the trans-European stratigraphic syntheses presented in papers by the Ziegler brothers, through the many field case histories (documented by

Europe in the Sixteenth century by A. Ortelius

geophysical logs and seismic sections), to esoteric accounts of palaeotemperature analysis, coal metamorphism, petrography of volcanic rocks, petrophysics of chalk, and intimate analyses of stratigraphic boundaries.

The oil companies who contributed papers to this volume are to be congratulated for their generosity in releasing such a wealth of information over which they have proprietary rights, and which they acquired at great expense and hazard, financial and human. Similarly, congratulations are due for the manner in which this volume has been produced. A large page size was sensibly chosen enabling ample display of maps, seismic sections and well logs. The figures are clear and uniformly drafted to a high standard. The quality of reproduction of photographs and seismic sections is also excellent. Each paper is followed by an account of the discussion which it generated, and the book is concluded by a subject index.

There is no doubt that the discovery of North Sea oil has been the biggest stimulus to British geologists since the advent of William Smith. This volume provides a landmark in the history of North Sea geological studies and may be the definitive text and sourcebook for more than a decade. It provides an excellent insight into the thought processes and extent of technical knowledge required by a practising petroleum geologist. This book may demonstrate to many geologists outside oil companies that the search for petro-

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leum requires a breadth of scientific knowledge, a clarity of thought, and an ability to synthesise and communicate complex data, that it might not have been thought possible could exist outside a university.

At £16 for 501 pages, this book is worth every penny. **R. C. Selley**

Petroleum and the Continental Shelf of North-West Europe. Volume 2: Environmental Protection. Edited by H. A. Cole. Pp. 126. (Applied Science: Barking, UK, 1975.) £8.

THE objective of the conference was to obtain authoritative statements from specialists in two fields: the effect of oil pollution on the marine environment and, from the oil industry, indications of how they operate offshore when drilling and developing oilfields, and the safety precautions taken to prevent spillage of crude.

The papers explaining how oil wells are drilled and fields developed and produced will, I fear, make heavy reading for the non-specialist to whom they should have been addressed. An overall impression of complex problems solved with sophisticated equipment and with considerable attention to preventing oil spillage is conveyed. Reading these papers, however, the non-specialist would have to be very persevering to understand what a blow-out is, how it occurs, who can prevent it and with what equipment.

Courtesy The Mansell Collection, London

The papers on oil pollution will be of much greater value to the non-specialist. They are no less technical than the oil industry papers but are neatly divided into the effects on Fisheries, Shore Life, Birds and Long Term Low Level Exposure in all areas.

The problems associated with analysing the effect of one pollutant, oil, in the marine environment are expressed in all papers, and admirably summarised in one, as follows: "Chronic pollution of marine waters by oil from numerous sources is widespread but hard to quantify. It is also difficult to specify its biological effects, since these can often be deduced only from laboratory experiments in conditions which are far from natural. In the field, it is rarely possible to exclude the effects of other pollutants or adverse natural conditions."

Two papers provide valuable data on the role of Government and the oil industry in oil spill clean up.

This volume will be a valuable reference to those interested in the problem of oil and its effects on the marine environment. **P. Selwood**

Geology of the North-West European Continental Shelf. Volume 1. By D. Naylor and S. N. Mounteney. Pp. 162. (Graham Trotman Dudley; London, 1975.) £6.50. Volume 2. By R. M. Pegrum, G. Rees and D. Naylor. Pp. 225. (Graham Trotman Dudley; London, 1975.) £8.50.

With the increasing level of effort now being put into the exploration and exploitation of the Continental Shelf surrounding the British Isles, a large number of non-petroleum specialists have become involved in the work. Bankers, lawyers, accountants, economists, and professionals in many disciplines are finding that an elementary understanding of the geology of the various areas in which their companies are working is a distinct advantage. And it is for such professionals that these books were essentially compiled.

It is not quite clear why the book is in two volumes particularly as both contain together only 380 pages. Nevertheless the division between the books is clear enough—the first dealing with relatively unexplored western basins of the Shelf, the second being confined to the North Sea area. If a division has to be made then this at least is a logical approach.

Assuming their intended market, both volumes tend to assume very little geological knowledge, but there are times when without it the layman will be stretching a little. Volume 1 begins by introducing the concept of plate tectonics in an attempt to place the

Biochemistry of hormone action

Biochemical Actions of Hormones. Volume 3. Edited by Gerald Litwak. Pp. xvi+415. (Academic; New York and London, August 1975.) \$36; £18.

READING this book straight through it becomes increasingly obvious that the real breakthroughs in the understanding of hormone action will only come when the tools provided by molecular biology and genetics are brought to bear on the problems. Unfortunately, not all the hormone-responsive systems currently under study are amenable to

British Isles in a regional framework. To a geologist the way in which this is done may seem a little glib but the message may get across nonetheless. By reference to geophysical and bottom sampling data acquired in each of the western basins together with a resumé of the inferred stratigraphy, a generalised picture is built up of the basins stretching from the Channel to the west of Shetland. The amount of information in each chapter varies with the amount of information published, but nevertheless for the audience intended there is sufficient data to give a reasonable idea of the variations in structure and rock type between each of the basins considered.

A more systematic treatment, however, with maps, cross sections and stratigraphic tables, would have enabled comparisons to be made rapidly, as would mention of the relationships between onshore and offshore geology, even in diagrammatic form. The account of the palaeogeography and the evolution of western Britain, brief though it is, seems somewhat misplaced. Indeed it might be said that the book tends on occasion to forget its audience. What will be useful to the layman, however, are the brief notes on how oil is trapped, and explanation of how a seismic survey is conducted and how a well is drilled. These are partially repeated in the second volume together with a glossary, but they perhaps bear re-reinforcement.

The second volume is concerned mainly with the North Sea and the information it contains is based on factual material, much of it released for the first time at the Conference in London last November, and which is drawn on freely. The stratigraphy is described systematically from the basement to the Tertiary system, and sadly the procedure of describing Basins is not adopted in this volume as it was in

these approaches and very often when these tools would prove useful they are not applied. The articles in this book serve to illustrate this point.

Molecular and genetic studies are obviously much easier where the gene product affected by the hormone can be identified; the chapter by Schimke *et al.* provides an elegant example of molecular biology being used to study ovalbumin induction in the chick oviduct. This chapter appears next to another (on the same topic) by Rosen and O'Malley which has an annoying amount of overlap. Indeed this is a general fault with the book: half of it is concerned with steroid hormones, and the central dogma of steroid action is repeated several times. But some of

the first. In many ways this method is a much more convenient one of describing an area which geologically has a very complex history.

The "field data sheets", which is effectively what they are, will be appreciated, however, by the layman as they condense a considerable amount of information very concisely. Each field is presented in a similar manner each having a map and cross section together with pertinent information on the field. The stratigraphic information which has been derived from studies of the fields is incorporated into the accounts of various geological systems and thus the fields are placed in their geological perspective. Because the book does not use the Basin approach of the first volume, however, the significance of the various discoveries (for example, the predominance of gas fields in the southern North Sea Basin) might go unnoticed.

It may be that it is too early in the history of the exploration of the Continental Shelf to attempt the synthesis which is presented in the second volume. There is still a great deal of information that remains confidential, and authors are unable to construct the geological cross sections and maps which would make all the difference to unravelling the history of these areas and which are so essential to an elementary understanding of the geology.

In many respects the two volumes are complementary, one deals with essentially untried areas the other with proven and established Basins. They both contain a considerable amount of compiled information—albeit very generalised. It is a matter of choice and of the reader's expertise as to whether one goes to the original text (the proceedings) or to this concordance.

J. R. V. Brooks

these articles are good. There is an excellent review of the action of oestrogens by Katzenellenbogen and Gorski, although it is somewhat marred by the primitive techniques used to analyse oestrogen-induced RNA synthesis.

There are three chapters dealing mainly with hormone receptors; that by Hollenberg and Cuatrecasas is a particularly useful introduction to peptide and catecholamine receptorology. One other chapter deals exhaustively with the glucocorticoid receptor and another discusses the evidence for a specific nuclear receptor for thyroid hormones.

Any genetic approach to the problem of hormone action presupposes that hormone-mediated events can be studied in cultured cells. The problems of establishing hormone responsive cells in culture is discussed in two chapters,

one by Armelin (which mentions some of the pitfalls) and a disappointingly brief chapter by Sato. In it he discusses the role of serum in cell cultures and comes to the conclusion that a whole new series of peptide hormones are about to be discovered.

The hardest task in reviewing this book is trying to decide who it was aimed at. Although some of the topics treated have not been reviewed previously, libraries are becoming full of books which serve as a vehicle for yet more reviews on the same topics. It might be more useful to have more shorter reviews with good references so that the reader has enough information to go to the relevant literature if he wishes. This book is, however, good of its genre and provides a relatively painless entry into some of the biochemistry of hormone action.

Robert Shields

Analysing sediments

Archaeological Sediments: a Survey of Analytical Methods. By Myra L. Shackley. Pp. 159. (Butterworths: London and Boston, Massachusetts, June 1975.) £7.00.

THIS book is a compendium of methods for the description and analysis of inorganic sediments laid down by wind and water, and for the handling of field and laboratory data. A large part of the book deals with particle size analysis and is a thorough and reliable guide to techniques in this field, but there is very little discussion of experimental errors, and a spurious precision of measurement is recommended, given the sampling strategy proposed. Interpretation of field observations and experimental results receives very little attention, and examples of the use of the methods are presented with uncritical acceptance of their suitability to the problems in hand and of the value of the results. Outside the field of traditional sedimentology, selection of sources and coverage of techniques is erratic, and misleading advice and information appears. In the confused discussion of the relationship between soils and sediments no mention is made of mineral alteration and secondary mineral formation; organic components of sediments, although their study is a separate field, surely require discussion as participants in the processes of sedimentation and postdepositional alteration. Most archaeological sediments are complex mixtures of mineral and organic materials of

diverse origin and history, laid down in intricate interstratification by people and animals as well as by the inanimate agencies, and their study demands a synthesis of methodology derived from many branches of field and laboratory investigation. This book understates the limitations of the contribution its methods can make in deriving information about human environment and behaviour from the deposits left by or related to human activity. It provides a useful short cut to the standard literature of sedimentology, but fails to provide the archaeologist or the scientist working with him with the help in formulation of questions, selection of appropriate techniques and interpretation of results which he badly needs.

Susan Limbrey

Small-scale meteorology

Climate in a Small Area: An Introduction to Local Meteorology. By Masatoshi M. Yoshino. Pp. xvi+549. (University of Tokyo: Tokyo; International Book Distributors: Hemel Hempstead, Herts, April 1975.) £16.

PROFESSOR YOSHINO has provided a successful textbook on small-scale climatology, an area which does not lend itself readily to systematic presentation. It is a multidimensional subject in which the range of physical variables—pressure, temperature, humidity, cloudiness, sunshine, rainfall, wind and others—are related to a comparable variety of physical features—hills and valleys, land and sea, rivers and lakes, trees and forests, towns and cities—all of which have effects which

differ according to the broadscale environment and to the geographical zone, season of the year, synoptic weather situation and so on. The subject is at the same time eminently practical and useful but unfortunately general principles do not get us very far and empirical studies for local application need to be made in every part of the world; the literature becomes ever more voluminous as many hundreds of worthwhile studies are published every year. How does one set about reducing such material to a textbook for a university course. Professor Yoshino is one of the few who have tried.

A short opening chapter on terminology is followed by 25 pages of 'History of Research', just enough to remind students that the subject is not a new one. In chapter 3 of 142 pages, climate is related to the surface, flat land, city, forest, seashore, lakeshore, riverbank, including some fundamental boundary layer theory which seems either too much or too little. A chapter of similar length concerns 'topography', here implying mountains, hills and valleys, and its relationship to climatic elements; the next is entitled 'Local Airstreams and Weather' and relates the weather (rather than the climate) with synoptic pattern, local fronts, and local winds, föhn, bora, and the like. The sixth and last shorter chapter is headed 'Local and Microclimate and Nature' which could mean anything but turns out to be just a taste of applied climatology, with geomorphology and plant ecology, and provides a place for a pet subject of the author, wind shaped trees.

A broad qualitative knowledge of meteorology and its basic principles is assumed and just occasionally mathematics finds a place. The Japanese book production is excellent and would do credit to the best English academic publishers although with sufficient minor misprints and infelicities in English to call for a little indulgence. It is a book to be acquired by every specialist library although the inevitable bias towards Japanese climate and Japanese literature makes it unsuitable as a coursebook outside Japan.

I have never taken readily to the textbook, however, which in its anxiety to acknowledge its sources reads at times more like an annotated bibliography. Nearly 1,000 different authors earn some 1,200 references and yet the selection from the much vaster world literature still seems almost arbitrary. A text for students which digests the material and is sparing with the 'credits' can be briefer and cheaper, more cogent, more instructive and certainly not so soon dated, although the scholarship may be less obvious.

R. C. Sutcliffe

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Edward Arnold

25 Hill Street, London
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Developmental controversy

Cell Cycle and Cell Differentiation.

(Results and Problems in Cell Differentiation: a Series of Topical Volumes in Developmental Biology, Volume 7.)

Edited by J. Reinert and H. Holtzer.

Pp. xiii+331. (Springer: Berlin and New York, 1975.) DM69; \$29.50.

As editors of *Cell Cycle and Cell Differentiation*, Reinert and Holtzer have put together chapters on some of the currently most interesting topics in developmental biology. This monograph can be roughly divided into two parts: the first four chapters deal with different cell types whose differentiation depends on a critical stage in the cell cycle, the remaining chapters either equivocally touch on the dependence of differentiation on the cell cycle itself, or argue that it may be irrelevant to differentiation.

The book begins with a chapter on myogenesis by S. R. Dienstman and H. Holtzer, the latter being currently one of the most prominent protagonists of the idea of the 'quantal cell cycle' as a key to understanding cellular differentiation. The next three chapters also echo the same theme of coupling of cell division or cell cycle to the differentiation of erythroid cells (H. Weintraub), of neuronal specificity (R. K. Hunt) and neurogenesis (C. H. Phelps and E. Pfeiffer). Those authors by and large agree with Holtzer's concepts but also urge caution before all the conclusions regarding lineage-dependence of differentiation are definitively accepted. The question of the mechanism of quantal cell cycle is however left unanswered, nor is it clear if such a cycle plays a 'causal' or a 'permissive' role in the expression of a developmental programme.

R. C. King gives a good account of the exploitation of genetics and hormones (particularly ecdysone) in evaluating the role of cell division during oogenesis in *Drosophila*, but avoids a clearcut conclusion about the causal role for the cell cycle or cell division. The book then takes a major turn with two short chapters by P. Lawrence and J. B. Gurdon who come out against quantal mitosis as essential for differentiation. Lawrence uses his work on pattern formation in larval and adult cuticle development in the insect, *Oncopeltus*, a process regulated by juvenile hormone, to argue that whereas asymmetric cell divisions may generate diversity it is not certain that symmetric mitosis leads to differentiation of daughter cells. Gurdon turns to his group's work on nuclear transplantation to argue that cell division

is not essential for all differentiative processes. He proposes that what may be important is cyclic condensation and swelling of chromosomes; the latter would pick-up a new set of cytoplasmic regulatory proteins during their postmitotic decondensation. It seems to me that there is room for Holtzer's quantal cell cycle idea as well as those of Lawrence's and Gurdon's and that these need not be mutually exclusive.

Four chapters depict the importance of studying differentiation in simpler organisms before attempting to interpret the more complex phenomena of animal cells. These include morphogenesis in the prokaryote, *Caulobacter* (N. B. Wood and L. Shapiro), plants in general (F. Meins, Jr), plant tumour formation as a differentiative process (A. C. Braun) and cell differentiation in *Neurospora* (R. E. Nelson, C. P. Selitrennikoff and R. W. Siegel). None of the authors of these chapters provide strong evidence for or against the idea of a quantal cell cycle as the basis of cell differentiation and hint that many morphogenetic changes can be dissociated from growth and cell division. Meins, after discussing mathematical analysis of several well known plant differentiative kinetic models, emphasises the well-known totipotency of differentiated plant cells which strongly suggests that cytodifferentiation results from epigenetic rather than permanent genetic modifications. Tsanev takes the reader back to animal cells with a detailed and fresh analysis of the very complex changes associated with hepatic regeneration, a very popular model for temporal analysis of functional changes and DNA synthesis but one which does not clarify the role of the cell cycle in differentiation and goes on to suggest that one should separately consider the activities of 'tissue-specific' and 'mitotic' genes. Th.W. Borun makes a plea for considering histones as important for early cell differentiation and not to regard them merely as 'glue' for keeping inactive genes repressed. He distinguishes the simple multiplicity of histones from the microheterogeneity generated by their phosphorylation, acetylation and methylation particularly of histones.

For students of developmental biology, *Cell Cycle and Cell Differentiation* will be most valuable for bringing together views of some of the most original workers in this field, but the controversy it generates about the essential role of quantal cell division may be more apparent than real. The monograph once again shows how far we still are from, or even how futile it may be to search for, a unified hypothesis for the oldest unsolved problem of biology.

J. R. Tata

obituary

Kasimir Fajans was born in Warsaw, Poland, May 27, 1887, and died in Ann Arbor, May 18, 1975. He went to school in Warsaw, to university in Leipzig, and then to Heidelberg where he did his Ph.D. work, and also won the Victor Meyer Prize (1909). He spent the next two years as a postdoctoral student at the Technische Hochschule in Zurich, where he became acquainted with Albert Einstein, and at the University of Manchester, where he worked with Lord Ernest Rutherford. After six years at the Technische Hochschule at Karlsruhe, he moved to Munich, and became the director of the Institute for Physical Chemistry in 1932. He first visited the United States in 1930 as the Baker Lecturer at Cornell University, and returned in 1936 as Professor of General and Physical Chemistry at the University of Michigan, where he remained for the rest of his career.

His major work was on radioisotopes, where he discovered the branching of radium transformation series in 1911, established the radioactive displacement laws in 1913, found the first isotope of the 91st element in the Periodic Table and discovered the precipitation rule of radio elements. Less well known, but equally fruitful, was his prolific research on other topics in chemistry. A partial list would have to include: the heat of hydration of gaseous ions, thermo-chemistry, chemical binding, volumetric analysis by adsorption indicators, theory of glasses, and the partial separation of D- and L-stereo-chemical isomers by asymmetrical catalysts. He was the author of five scientific books, and remained active long after his formal retirement, the last of many awards given to him, the Gold Award for 1975 of the Affiliate Council of the Engineering Society of Detroit, was bestowed only a few months before his death.

Professor Fajans married Salomea Kaplan, in 1910. She survives him together with their two sons Edgar W. and Stefan S., four grandchildren and two great grandchildren.

Thomas M. Dunn

Like many men of modest physical stature, **Hamilton Hartridge** who died on January 13, 1976, at the age of 89, was a dynamo. He used to produce

ideas at the rate of a normal person's heart-beat, a faculty which dove-tailed with his nineteenth century many-sidedness, but ultimately led to his professional evaporation.

After completing his medical studies he stayed at King's College Cambridge till 1926. It was there that he did his best scientific work. He collaborated with Roughton in the classical analysis of the composition of blood in relation to various levels of oxygenation: the study was furthered by Hartridge's ingenious invention of the reversion spectroscope, suitable for an estimation of the movement of haemoglobin bands. He suspended silk threads from the ceiling of his room at distances just greater than a bat's wing-span, and showed that, though the bats flew around, not a single thread was torn. He provided an optical system enabling Rutherford and Geiger to get on with their experiments counting α -particles. Later he buttressed Helmholtz' theory of hearing. He applied physical principles to the theory of visual resolution in the early twenties, was elected to the Royal Society and appointed subsequently—if not consequently—to the chair in physiology at Barts in 1927. This he occupied till 1947.

Paradoxically, his name is best known in the world of vision undoubtedly because of his literary rather than his scientific output. His crucial experiment on accommodation in the cat is forgotten. But his quixotic attacks on the validity of Young's trichromatic theory of colour vision raised enough dust for even some present-day authors deeming it necessary to flick a duster. Hartridge's lively brushes at the Physiological Society and the Colour Group of the Physical Society (as it then was) intrigued even laypersons.

Explicitly presented in a volume of Transactions of the Royal Society, affectionately known as the Yellow Peril, his polychromatic theory is alien to British sensory physiology in being unquantified and therefore untestable. The Medical Research Council set up a Vision Research Unit in 1947 under Hartridge's direction so that he might have a chance to prove his ideas. He failed. Yet he managed to attract a handful of people who have directly and indirectly stimulated vision research in the last thirty years both in this country and abroad. This should not be forgotten even though it is unlikely to be engraved on any stone.

R. A. Weale

Dr R. W. B. Nurse, a former Head of the Materials Division at the Building Research Station and a world authority on cement chemistry, died on November 13, 1975, aged 62. He received his degree in Physics from London University in 1934 and began at the BRS, which he joined in 1932, his fundamental work on inorganic building materials including the thermodynamics of multicomponent systems. Early in his career he invented with F. M. Lea (later Sir Frederick Lea) the Lea-Nurse apparatus for determining the specific surface of powders by an air-permeability method. He made major contributions to Portland and slag cement chemistry, for which he was awarded the D.Sc., London, in 1954, and contributed principal papers to the 4th, 5th and 6th International Symposia on the Chemistry of Cement. He was probably best known for his work on the manufacture of cement from impure limestone which led to the setting up of a thriving cement industry in Uganda, the first anywhere in the world to use phosphatic limestone. His work was recognised in 1963 by the award of the Sir George Beilby Medal. He also initiated at BRS studies of fibrous composites and crystallised glasses. In 1965, in recognition of his outstanding research abilities he was awarded special merit promotion to Deputy Chief Scientific Officer, which enabled him to reduce his administrative responsibilities and to concentrate on research. Between 1965 and his retirement in 1973, he continued his work on fibrous composite materials, contributing to the understanding of the properties and fracture mechanics of glass fibre cement composites in particular. He had been President of the Mineralogical Society and was an honorary member of the Permanent Committee of RILEM (Réunion Internationale des Laboratoires d'Essais et de Recherches sur les Matériaux).

Ron Nurse was a man of very wide-ranging interests and talents: he took a particular interest in the training of young people, for instance as a member of the Board of Governors in local schools and colleges. Among his many qualities his generosity of mind, modesty and his willingness to help others were outstanding. He leaves a wife, a son and a daughter by whom, as well as by his colleagues and friends, he will be greatly missed.

Witold Gutt

announcements

Appointments

Sir Denys Wilkinson, Professor of Experimental Physics at the University of Oxford, has been appointed Vice-Chancellor of Sussex University.

Professor John B. Goodenough, Leader of the electronic materials group at M.I.T., has been appointed Professor of Inorganic Chemistry at the University of Oxford.

Awards

The Geological Society of London has announced the following awards for 1976:

The **Wollaston Medal** to **Sir Kingsley Dunham**, Foreign Secretary of the Royal Society, an authority on mineral occurrences in Northern England.

The **Murchison Medal** to **Professor R. A. Howie**, King's College, London, for his research on pyroxenes and other minerals and his contribution to teaching.

The **Lyell Medal** to **Mr W. B. Harland**, University of Cambridge, for work in Spitsbergen, contributions to stratigraphy and the geology of tillites and work on orogenesis.

The **Prestwich Medal** to **Professor W. W. Bishop**, Queen Mary College, London, for his studies of mammalian palaeontology and the stratigraphy and geomorphology of Miocene-Pleistocene deposits in East Africa.

International meetings

March 1, Deadline for **Experimental Use of Algal Cultures in Limnology** to take place on October 26-28, in Sandefjord, Norway (Norwegian Institute for Water Research, P.O. Box 333, Blindern, N-0slo 3, Norway).

March 25-26, A symposium on **The Zoological Society of London 1826-1976 and Beyond**, to be held at the Meeting room of the Zoological Society (Assistant director of Science (UM), Zoological Society of London, Regent's Park, London NW1 4RY, UK).

March 30-April 2, **Sixth Conference on Molecular Spectroscopy**, University of Durham (Institute of Petroleum, 61 New Cavendish Street, London W1M BR, UK).

March 30-April 2, **The Synapse**, St Andrews, Scotland (Dr G. Cottrell, The Wellcome Laboratories, The Gatty Marine Laboratory, St Andrews, Fife, UK).

Person to Person

Joseph Lister, a new biography. I wish to consult letters, diaries, related materials held by friends, former patients, family, especially descendants of Lister's sisters, Isabella Sophie Pim and Jane Harrison, and of James Syme, the Scottish surgeon. Richard B. Fisher, 26 St Paul's Road, London, N1. 01-226 2765.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

March 31, Deadline for attendance at an **International Symposium on Migraine**, London (The Director, The Migraine Trust, 23 Queen Square, London WC1N 3AY, UK).

March 31-April 2, **Intraocular Foreign Body and Metallosis**, organised by the German Ophthalmological Society, Cologne (Professor Dr H. Neubauer, Universitäts-Augenklinik D 5 Cologne 41, Joseph-Stelzmann-Strasse 9).

April 1-2, **A Symposium on Diabetes and Other Endocrine Disorders During Pregnancy and in the Newborn Infant**, New York City (Professor Maria I. New, Cornell University Medical Center, 525 East 68th Street, New York, N.Y. 10021).

April 5-8, **mRNA: The Relation of Structure to Function**, Gatlinburg, Tennessee (Elliot Volkin, Chairman, Organising Committee, 1976 Biology Research Conference, P.O. Box Y, Oak Ridge, Tennessee 37830).

April 5-6, **Microscopy of Organic Sediments**, Oxford (The Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford OX4 1AJ, UK).

April 5-8, **Anglo-Dutch Wood Anatomy Meeting**, Oxford University (The Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford OX4 1AJ, UK).

April 5-9, **Fifth European Ophthalmology Congress**, Hamburg (The Congress Secretariat, c/o Holland Organising Centre, 16 Lange Voorhout, The Hague, The Netherlands).

April 5-9, **EMBO Workshop on Thermodynamics and Calorimetric Studies on Biological Systems**, Santa Mar-

gherita Ligure, Genoa (Dr Giovanni Rialdi, c/o Centro Chimica Fisica di Macromolecole Sintetiche e Naturali, Consiglio Nazionale delle Ricerche, Istituto di Chimica Industriale, Via Pastore, 3-16132 Genova, Italy).

April 5-9, **The Annual Chemical Congress of the Chemical Society and the Royal Institute of Chemistry**, Glasgow (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN, UK).

April 6-8, **Components of Protein Synthesis**, Cambridge (Dr A. R. Fersht, M.R.C. Laboratory of Molecular Biology, University Postgraduate Medical School, Hills Road, Cambridge CB2 2QR, UK).

April 6-8, **Advances in Mechanisms of Drug Metabolism and Species Differences in Drug Metabolism—from Microbe to Man**, Guildford (Dr L. J. King, Department of Biochemistry, University of Surrey, Guildford, Surrey GU2 5XH, UK).

April 6-9, **Biology and Systematics of Colonial Organisms**, Durham (Dr B. Rosen, Department of Palaeontology, British Museum (Nat. Hist.), London SW7 SBD, UK).

April 7-9, **A Symposium on Endocytosis and Intracellular Digestion**, organised by the British Society for Cell Biology/Lysosome Club, Nottingham (Dr T. R. Ricketts, Department of Botany, School of Biological Sciences, University of Nottingham, Nottingham NG7 2RD, UK).

April 8-9, The British Institute of Radiology announces its **34th Annual Congress and Scientific Exhibition**, London (The General Secretary, The British Institute of Radiology, 32 Welbeck Street, London W1M 7PG, UK).

April 8-9, **Moisture in Solid Materials in the Process Industries**, jointly organised by SIRA and The Koninklijk Instituut van Ingenieurs (Netherlands), Delft (Mrs R. G. Keiller, SIRA Institute Ltd, South Hill, Chislehurst, Kent BR7 5EH, UK).

April 8-9, **The Ordinary Meeting of the Royal Astronomical Society**, Manchester (The General Secretary of the Royal Astronomical Society, Burlington House, London W1V 0NL, UK).

April 30, Deadline for abstracts, **The Stratosphere and Related Problems**, Logan, Utah, on September 15-17 (Dr Wesley T. Huntress, Jr, Executive Secretary, Jet Propulsion Laboratory, M/S 183-401, 4800 Oak Grove Drive, Pasadena, California 91103).